PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 19 September 15, 1933 Number 9

A TREE-RING CHRONOLOGY FOR THE RIO GRANDE DRAINAGE IN NORTHERN NEW MEXICO

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Communicated July 25, 1933

In 1929 Doctor A. E. Douglass announced the establishment of a tree-ring chronology in the Southwest back to A. D. 700, by means of which roof-beams from prehistoric Pueblo ruins could be dated.[1] The area which this chronology covered is in northern Arizona, northwestern New Mexico, southwestern Colorado and southern Utah, within the watersheds of the Little Colorado and San Juan rivers.[2] Attempts to carry the dating into the drainage area of the Rio Grande in northern New Mexico by direct comparison with material of this chronology were made as early as 1922; but the results were unsatisfactory, and the dating of ruins in the "Rio Grande area" remained an important problem. This paper is an announcement of some preliminary results of the application of Douglass' method of dating to this area.

Douglass has described the method of dating in several publications.[3] Salient points may be summarized as follows:

> Sequences of wide and narrow annual rings characteristic of given periods of years are present in certain trees and can be recognized from tree to tree and over large areas. These sequences are attributed to environmental changes climatic in character. In the semi-arid Southwest the dominant factor is recognized as precipitation.
>
> The fundamental principle of the method lies in the cross-identification of these sequences in a number of specimens, by which procedure the annual identity of each ring is established. This may be expressed as a procedure of matching climatic patterns of ring-widths, in performance of which each specimen assists in checking (and in some cases, correcting) the records of the others.
>
> By cross-identifying specimens of different time series which overlap from the present into the past, a long chronology can be built and sequences of past times dated.

As the results of fresh attempts made in the spring of 1931 to date "Rio Grande" material by the chronology of northern Arizona and adjacent regions were subject to considerable doubt,[4] the problem was approached by the writer with the intent of erecting an independent chronology by carrying the dating back from living trees within the area. Col-

lections of living and archaeological material, in the form of increment cores, wood-sections and charcoal, were made from the vicinities of Taos and Gallina on the north to Gran Quivira on the south, and from the western side of the Jemez mountains and the Sandia-Manzano chain eastward to the Sangre de Cristo range and mesas adjacent to the south. The cultural span covered was from ruins of the Pueblo III horizon of Southwestern archaeology through later Pueblo and Spanish buildings to the present. From this and the material collected previously within the area by Doctor A. V. Kidder, Doctor Douglass and the writer, a chronology back to A. D. 1205 has been erected, and ruins dated.

Yellow pine (*Pinus ponderosa* Laws.), piñon (*Pinus edulis* Engelm.), and Douglas fir (*Pseudotsuga taxifolia* Britt.) were the species used. Yellow pine was the chief one, comprising about three-fourths of all the dated archaeological specimens; piñon, slightly less than one-fifth; and Douglas fir, the remainder. Datable collections from living trees were made in a similar ratio. The same climatic patterns are apparent in all three species; that is, the annual rings of any one species could be dated by the relative width criteria of those of the other two.

78 per cent of the chronology is covered, at any one year, by at least 25 specimens. From 1290 to the present, no one point is verified by less than 15. Excepting the nineteenth and twentieth centuries, covered by some two to three hundred specimens in progressive frequency toward the present, the largest number at any one point is 79. Preceding 1290 the number dwindles from 14 to 5. The few records of the early twelve-hundreds are, however, from different localities and habitats: the Pajarito plateau, the Rio Ojo Caliente drainage and the Galisteo Basin; and are of two species: yellow pine and piñon.

The chronology thus erected has many distinctive years in common with Douglass' chronology for northern Arizona and adjacent regions, and a time corroboration is evident at many points throughout the mutual seven and a quarter centuries of tree-ring records (cf. footnote 4).

Below are listed a series of sites, dates of which are announced at this time. Site numbers of the Archaeological Survey of the Laboratory of Anthropology are given in parentheses. ± 5 or a smaller number means that the last few rings grown by the specimens are apparently not present, or that they are too compressed to be identified accurately to the year; thus, 1310 $\pm$ 5 means that the cutting date of a beam or beams is within five years of 1310. Where the specimens are decidedly fragmentary and the cutting dates involve estimations of more than ± 5 years, the date of the last ring present plus the symbol X is given before the estimated cutting date.

Pueblo III

Water Cañon Ridge ruin (545); 1310 $\pm$ 5. A plaza-type site on the Pajarito plateau.

Lamy (27); 1275 + X, estimated 1300 ± 10. One of a group of black-on-white sites about half a mile southwest of Lamy.

(309); 1310 ± 5. A site in the Galisteo Basin about two and a half miles north of the ruins of San Cristóbal.

Pueblo IV

Posí (Poseuinge) (632); 1331 + X, estimated 1375 ± 25.

Hupobi (Homayo) (380); 1320 + X, estimated 1375 ± 25.

(251); 1390. A site about 2 miles east of Glorieta with early pottery types of Pueblo IV, along with those of Pueblo III. The date is correlated with the early development of Pueblo IV in the region.

Pueblo Largo[5] (183); 1435.

Frijolito (78); 1447.

Tyuonyi (82); 1440 + X, estimated 1460 ± 15.

Puyé (47); 1507 to 1565 ± 3.

Tshirege (170); 1460 + X, estimated 1480 ± 10; to 1574.

Unshagi (123); 1572 to 1594.

Tunque (240); 1431; 1490 ± 5.

San Cristóbal (80); building X of Nelson[6]; 1427 to 1442 ± 5.

Pecos (625); 1348 to 1612.

(136); 1657. A large, unnamed Jemez ruin between Vallecito Viejo Creek and San Juan Cañon, Rio Jemez drainage.

These dates are not necessarily the earliest building dates of the sites, nor do they indicate the whole time of occupancy. They indicate merely that trees were cut for roof-beams at the dates listed.

This communication is released following an examination and check of the chronology by Doctor Douglass, to whom grateful acknowledgment is made. The building of the chronology was made possible by a research project initiated in June of 1931 by the Laboratory of Anthropology for the primary purpose of dating Pueblo ruins and cultural material in the Rio Grande drainage in northern New Mexico. Shortly thereafter Phillips Academy, Andover, and Carnegie Institution of Washington, at present collaborating in a study of the results of the excavations at Pecos, coöperated in the project with regard to research upon the beams from that site. Credit is also due several institutions and many individuals who have contributed material or otherwise given valuable assistance. It is regrettable that full acknowledgment cannot be made at this time.

Detailed papers on the chronology and problems involved are planned for future publication.

[1] "The Secret of the Southwest Solved by Talkative Tree-Rings," *National Geographic Magazine*, **56** (6), 737–770, December (1929).

[2] Subsequent work has extended this area in places beyond the limits of the two watersheds.

[3] Most completely in "Climatic Cycles and Tree-Growth," *Carnegie Institution of Washington*, Pub. 289, **1** and **2**, Washington (1919) and (1928). Shorter papers in many publications. See bibliography, op. cit. Also: reference cited footnote 1;

"Tree Rings and Their Relation to Solar Variations and Chronology," *Ann. Rep. Smith. Inst.* (1931), 304–312, Washington (1932).

[4] Some of the doubtful dates obtained at this time later proved correct.

[5] The Pueblo Largo of N. C. Nelson, "Pueblo Ruins of the Galisteo Basin, New Mexico." *Anthro. Pap. Amer. Mus. Nat. Hist.*, 15 (1), 68 (1914).

[6] Nelson, op. cit., 59–63.

SOME OBSERVATIONS ON THE ARCHEAN METAMORPHICS OF THE GRAND CANYON

By Ian Campbell and John H. Maxson

Balch Graduate School of the Geological Sciences, California Institute of Technology. Publication No. 88

Read before the Academy, Monday, April 24, 1933

Although the rocks exposed in the inner gorge of the Grand Canyon present one of the best Archean sections in the United States, and although their occurrence has been known since the time of Powell's classic exploration in 1869 and 1874, relatively little attention has been given to their study. This may be due in part to the complexity and inaccessibility of the rocks themselves, and in part to what may have seemed the superior attraction of the later formations, exposed at higher levels in the Canyon walls.

The study of this terrane holds special interest, however, not only from the point of view of igneous and metamorphic petrology, but also from the fact that it involves problems concerning the early history of the globe. The writers are indebted to the Carnegie Institution of Washington and the U. S. National Park Service for the opportunity to undertake this study.

The purpose of this preliminary paper—preliminary because most of the data are based upon the field work alone, and not upon intensive laboratory investigations—is to present some of the more significant findings in regard to the metamorphic rocks, without attempting much general discussion of the relationships involved. Most of our present knowledge of the Archean is due to Noble[1] who described the principal rock types and indicated, in a broad way, their distribution.

This study has brought out new evidence which confirms the earlier judgments of Walcott[2] and of Noble concerning the sedimentary nature of the schists. Within the area studied, the writers have become convinced of the sedimentary origin of the great body of rocks to which the name "Vishnu Schist" (sensu strictu) may be applied. Summarized briefly, the evidence falls under two headings:

STRUCTURAL	PETROLOGIC
Cross-bedding	Quartzites
Stratification	Calcareous beds
Lenticular bodies	Iron formation

Thus in Lone Tree Canyon there is well exposed a section of quartz mica schists, in which such unequivocal sedimentary features as cross-bedding and quartzitic sandstone lentils are preserved. (See Figs. 1 and 2.)

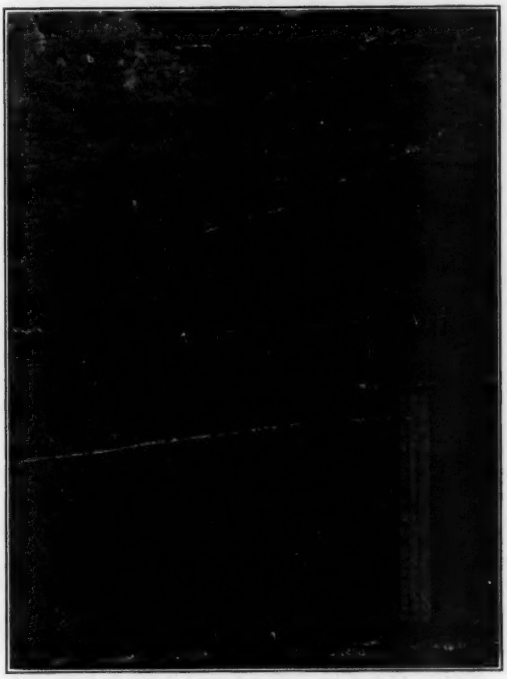

FIGURE 1

Quartz-sericite schist exposed in the floor of Lone Tree Canyon. In connection with the cross-bedding note that the sharper truncations are toward the west, indicating that the younger beds lie in that direction. The scale is six inches long.

It is interesting to note that within a few hundred feet of this least metamorphosed Archean exposure, there occurs a garnet-staurolite schist, associated with a granitic intrusion. This relationship is an indication that at least a part, and perhaps much, of the higher degrees of metamorphism found in the rocks in this area may be due to contact rather than to regional effects.

Quartzites occur at many places in the section, and while the majority are very thoroughly recrystallized, some preserve evidence of stratification.

An interesting discovery is that of thin beds and small lentils of a lime-silicate rock, composed of garnet, diopside, epidote and calcite—an as-

FIGURE 2

Another view of the quartz-sericite schist in Lone Tree Canyon, showing sandstone lentil below the rule. Note that schistosity generally parallels the bedding. The white streaks are quartz veinlets. The scale is six inches long.

semblage typically developed in the metamorphism of impure calcareous rocks. These occurrences, which are found in both Horn and Pipe Canyons, probably represent original calcareous lenses of a shale.

Also in Pipe Canyon, there is a rock classed as "iron formation" which on analysis is seen to be very similar to itabirite, the ferruginous quartzite of Pre-Cambrian age forming the iron ores of Minas Geraes.

There can be no question as to the original character of the types just mentioned. But the origin of many of the schists might be obscure, were

it not for their association and inter-bedding with the types of undoubted sedimentary character, for igneous invasions have greatly complicated the picture. Thus not infrequently it is possible to trace, by almost imperceptible gradations, a quartz-mica paraschist into a typical granite. In these cases the grain of the schist becomes gradually coarser, the mica orientation tends to disappear and the proportion of feldspar increases. It is difficult, if not impossible, in such cases, to mark a contact or to assign relative proportions to the two end types. Certainly the processes of assimilation, replacement, granitization, etc., have played a major rôle in the development of many of the types here found, and even in the case of the most typical Plutonic igneous rocks, there is the possibility that much of their substance has been derived from earlier sediments.

Assuming a sedimentary origin for the Vishnu schist, it is natural to inquire next into the general conditions of its formation—its environment of deposition. Not much data applicable to this question have yet been accumulated. A few facts are pertinent, however. First, there is the apparent absence from the section of conglomerates on the one hand, and limestones on the other. Second, there is the great thickness of the section. It is entirely possible that the distance now measured across the strike will have to be reduced considerably, if either isoclinal folding or reverse faulting, with duplication of portions of the section, can be proved. But even if the section were to be reduced one-half (an extreme figure) it would still be measured in tens of thousands of feet. Third, throughout this thick section, sedimentation appears to have been rather uniform. The sediments were dominantly sandy clays. Fourth, the few examples of cross-bedding observed indicate marine rather than aeolian or fluviatile deposition. All things considered, therefore, these Archean sediments may be assumed to have been laid down in a shallow, subsiding geosyncline. They are accumulations of the neritic zone, and may perhaps be compared to the arenaceous shales of the Belt series of the northern Cordillera or even—to bring the comparison closer—to the non-calcareous sediments of the overlying Grand Canyon series (Proterozoic) represented by such formations as the Shinumo quartzite and the Hakatai shale.

[1] Noble, L. F., "A Reconnaissance of the Archean Complex of the Granite Gorge, Grand Canyon, Arizona," *U. S. G. S.*, Prof. Paper 98–I (1916).

[2] Walcott, C. D., "Pre-Carboniferous Rocks in the Grand Canyon, Arizona," *Amer. Jour. Sci.*, **26**, 437 (1883).

A PRELIMINARY SURVEY OF THE SPECIES OF ZOSTERA

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Communicated August 11, 1933

The genus *Zostera*, comprising the Eel-grasses, is credited by Ascherson and Graebner, in 1907 (Engler u. Prantl, Pflanzenreich, IV, II, lief. 31, pp. 27–32), with 7 species, 2 inhabiting the North Atlantic, 2 the North Pacific, 1 the southwestern Indian and 3 the South Pacific oceans. They retained Ascherson's divisions ("sections") of *Alega*, with 2 species, and *Zosterella*, including the remaining 5. No additional species have been proposed since except the 3 of Miki (*Bot. Mag. Tokyo*, **46**, 774–788 (1932)), bringing the total number up to 10, or probably 9 which can be retained as distinct. In my own attempts to obtain a knowledge of the species of the group, 2 austral species (1 from South Africa and 1 from New Zealand) have manifested themselves as undescribed, while 3 other austral forms, possibly to be segregated, must remain, as yet, uncertain.

The critical characters of *Zostera* extend throughout the whole plant and, taken in conjunction, clearly delimit each species. They are both vegetative and reproductive, partly morphological and partly histological. It seems desirable to pass them in brief review before attempting to enumerate and characterize the species.

The stems are of two types, the perennial vegetative and the annual reproductive, both present in most species. The perennial vegetative stems are rhizomes, prostrate and rooting in all the best known species, yet in the Chilean and in at least 1 of the Tasmanian species they are largely erect or ascending and rootless. The annual fruiting stems are erect branches of the prostrate perennial rhizomes, are rootless, perishing at the end of the flowering and fruiting season. The behavior of both the rhizome and the erect stem has been outlined elsewhere (Setchell, *Univ. Calif. Publ. Bot.*, **14**, 420–421 (1929)).

The internodes of both types of stem show, for each species, the same arrangements of tissues. They were particularly investigated by Sauvageau (*Jour. Bot.*, **5**, 59–68 (1891)). The details and differences brought forward by Sauvageau have been verified and confirmed. His material of *Zostera Muelleri*, however, did not properly belong to that species but to a species identical with, or more probably closely related to, *Z. tasmanica* (a group of species or forms to be discussed later on). In all the species of *Zostera* there is a central "vascular" cylinder traversing the internode. In all the species there are also peripheral (lateral) "vascular" bundles in the flanks of the somewhat or more considerably compressed internodes. In the "*tasmanica*" group, each flank has 2–5 lateral bundles, while each of the

other known species (including the true *Z. Muelleri*) has only a single lateral bundle in each flank. The "*tasmanica*" group is, therefore, set off from the other *Zosteras* by the more numerous lateral bundles. The other species (outside the *tasmanica* group) have farther differentiation into those with the fibre strands scattered throughout the outermost portion of the outer cortical layers (extending to the epidermis) as in the species of the *Alega* Section (*Z. marina*, *Z. caulescens*, *Z. asiatica* and *Z. caespitosa*), while in the *Zosterella* section (excluding the *tasmanica* group), the fibre strands are situated farther in, leaving the outer cortex (in narrower sense) free from them and forming a broader or narrower zone of sparser or more closely placed bundles about two-thirds of the way between the central cylinder and the epidermis.

Members of the *Alega* section have 2 lateral vascular bundles in the outermost layers of the outer cortex. Members of the *Zosterella* section have also only 2 lateral vascular bundles but *Z. tasmanica* and its immediate allies have 4 to 10 lateral vascular bundles. All the members of the *Zosterella* section (excluding the *tasmanica* group) have the fibre bundles scattered in inner layers of the outer cortex while in the other species they are in the outermost layers of the outer cortex. A transverse section of an internode (preferably of a rhizome) at once indicates the plant as to membership in 1 of 3 groups.

The foliage leaves are essentially similar in all parts of the plant, only the *praefolium* or first leaf of a shoot or branch presenting fundamental peculiarities. Sauvageau (*Ann. sci. nat. Bot.*, 7 ser., **13**, 126–153 (1891)) has made comparative studies of the leaves. All typical leaves have a sheathing base (sheath) extending both into a short outer structure (ligule) and an inner elongated blade (lamina). The sheath is closed (or "fused") in species of the section *Alega*, while it is open (or "split") in all other species of *Zostera*. The sheath is more papery, or more or less fibrous in one or another species. The ligule differences are sometimes of service in separating species. The blade is usually flat and smooth. In an undescribed variety (or form?) of *Z. marina* it is sulcate. In cross-section, the blade may be long elliptical (most species of *Alega*) or more oblong (most species of *Zosterella*).

The number of longitudinal primary nerves ("vascular bundles") of the blade varies from 3 to 11, according to the species, the larger number, 7 to 11, being confined to species of *Alega* while *Zosterella* shows only 3 (seemingly only 1 when the two lateral nerves are very close to the margins) except in *Z. capricorni*, which typically has 5 (at least below). There is some variation in number even in the same plant and particularly where the rhizomal leaves are much broader than are those of the erect annual stems in the species of the section *Alega*.

An important leaf characteristic is the number of longitudinal "inter-

stitial" nerves or fibre bundles. Between the central vascular bundle or "primary nerve" and each of the two adjacent lateral nerves, the number of interstitial nerves varies somewhat in the same species, but within limits distinctive of the species. The number varies from 2 to 9. Taken in connection with other characters, this number is of diagnostic importance.

Finally, the tips of the mature true foliage leaves are characteristic of the individual species. This has been indicated by Ascherson and Graebner (loc. cit.) and enlarged upon by Sauvageau (loc. cit., 2). The differences are not readily expressed in words, but an attempt will be made to express the tip character under the notes on the individual species The leaf margins, apart from the immediate apex, are normally smooth, but in *Z. caespitosa* they are, at times, distinctly although minutely ciliated.

Sauvageau (*Ann. Sci. Nat.*, ser. 7, **13,** 141, 146 (1891)) has called particular attention to the existence of the *squamulae intravaginales*, structures arising from the outer tissues of the internodes near their bases, but not directly attached to the leaves either above or below them. Agnes Arber (*Monocot.*, 94–96 (1925)), discussing the "leaf-skin theory," seems inclined to regard them as belonging to the leaf above, rather than below them. In *Z. marina* they are usually in fours, but Sauvageau says that the number is variable. In all other species they are in pairs.

The individual inflorescences consist of a basal internode (or joint), bearing a leaf, whose sheath is more or less modified into a spathe (enclosing the spadix or flower axis) and prolonged into a terminal, more or less shortened blade, which is earlier or later (in anthesis or fruiting) deciduous. The spadix may possibly represent a single internode but more logically a succession of fused internodes. It is non-green (white), flattened, smooth on the side toward the enveloping spathe, but on the other (dorsal?) side is broadly channelled and bearing a zig-zag succession of flowers (from about 7 to 13 or even 15), if one supposes each flower to consist of a single stamen and its opposite pistil. In *Zosterella*-species there is a retinaculum (bract?) opposite each stamen (arising within the elevated margin of the spathe), but in *Alega*-species retinacula are lacking or possibly at times vestigial in some of the lowermost flowers. The lowest flower and the uppermost 1–3 flowers are usually small and non-functional, usually reduced to the pistillate member only. The presence or almost complete absence of retinacula separates the members of the *Alega* section from those of *Zosterella* (see, however, Sauvageau, *Ann. Sci. Nat.*, ser. 7, **13,** 149, 150 (1891)) and the differing outlines and dimensions of the retinacula may be used to distinguish the species.

With the ripening of the seeds, other characters appear as to size of the mature seed and especially of seed coat characters. The seed coat of *Z. marina* is usually described as "sulcate" (e.g., Ascherson and Graebner, loc. cit., 30), but the expression "costate" seems better, since there are dis-

tinct longitudinal ridges produced by the upturning of the ends of the elongated and horizontal outer cells of the testa (see Jensen, *Bot. Tidsskr.*, **17**, 163, Fig. II (1888), and Setchell, loc. cit., 398, Fig. 2). The only other costate testa in *Zostera* is found in the seeds of *Z. caespitosa*, but the costae are rounded and blunt in this species. The testae of the other species of *Zostera* are devoid of costae, although they may appear "striate" when dry. The outer walls of the outermost cells of the testa are usually smooth, but in that of *Z. caespitosa* they are delicately, minutely and closely verruculose.

SYNOPSIS OF SPECIES

Section 1. *Alega* Aschers

Retinacula absent or occasionally vestigial; leaf-sheaths closed; large plants with relatively broad to very broad leaves with intermediate nerves; rhizomes with 2 lateral bundles and fibre strands in outermost cortex; spathes slightly if at all broader than the peduncular internode. Four boreal species.

A. TESTA LONGITUDINALLY COSTATE

1. *Zostera marina* L.

Leaf with broad apex, pointed or mucronate, primary nerves 5–7 (3–9), interstitial nerves "numerous" (usually 4–7); sheaths thin membranaceous; squamulae intravaginales normally 4; testa with sharp elevated longitudinal costae (16–25). North Atlantic Ocean (Eur., No. Amer.); North Pacific Ocean (No. Amer., N. E. Asia).

Varieties *stenophylla*, Asch. and Graeb., *angustifolia* Hornem., *typica*, and *latifolia* Morong seem to represent different degrees of development and robustness of leaves, inflorescences and rhizomes, possibly simply reversible reactions to one or more of the environmental characters. A narrow-leaved plant from "Reikiavik," Iceland (Herb. Kew, sheet 5, coll. Babington), differs from other forms of *Z. marina* in having a narrow, deep, cleft-like emargination at the apex. If this be found general and normal, this type of plant may ultimately find independent specific rank.

f. *sulcatifolia* f. nov.

Z. marina foliis in longitudine profunde sulcatis. False Bay, San Diego, California, Harold E. and Susan Thew Parks, Jan., 1930.

The "furrow-leaved" Eel Grass may be only a peculiar ecophene, the leaves affected being foliage leaves and in many specimens, in a somewhat limited area now unfortunately rendered uninhabitable to *Zosteras*, or, it may be all the specimens represent clones of a sudden variant. It seems to be a form well worthy of a name and a record.

2. *Zostera caespitosa* Miki

Leaf with apex broadly and shallowly emarginate through broadly concave-degeneration, margins at times finely cilate, primary nerves 5–7, interstitial nerves about 6, sheaths long persistent and thick parchment-papery; rhizomes short, ascending; squamulae intravaginales 2; testa with about 18–20 blunt, broad costae, epidermal cells minutely and closely verruculose.

North Pacific (Japan).

B. TESTA NOT LONGITUDINALLY COSTATE

3. *Zostera asiatica* Miki

Leaf with apex retuse, desquamation slight, primary nerves 9 (7–11), interstitial

nerves 8–9, sheaths thin membranaceous; rhizome stout, horizontal; squamulae intravaginales 2; testa smooth; embryo without anthocyanin blotches.

North Pacific (Japan, Corea).

This and the next are very robust species with leaves up to 15 mm. broad.

4. *Zostera caulescens* Miki

Leaf with apex broad, mucronate, very slightly desquamate, primary nerves 9 (7–11), interstitial nerves 6–7, sheaths thin membranaceous, ligule stout; rhizome stout, erect stem much elongated, fertile below; squamulae intravaginales 2; testa smooth, indistinctly longitudinally striate when dry; embryo with anthocyanin blotches.

North Pacific (Japan, Corea).

A robust species, with leaves broad (14 mm.), conspicuous erect stem, fertile below, and with smooth testa.

Section 2. *Zosterella* Aschers

Retinacula regularly present on all except the more or less aborted lowest and uppermost flowers; leaf sheaths open; testa ecostate.

Low or moderately tall species with 3 (appearing often as 1) nerved leaves (except in no. 5); squamulae intravaginales 2; rhizomes with 1 lateral bundle on each flank and fibre strands in inner portion of cortical layer; spathes from slightly to decidedly broader than the peduncular internode. *Z. nana* and *Z. japonica* boreal, the other species austral.

A. Fertile Erect Stem Normally Much Elongated

5. *Zostera capricorni* Aschers

Leaf with apex roundish-flattened, sharply but irregularly, at times sparsely denticulate, primary nerves 5 (3), interstitial nerves 5 (2, 3, 4), sheaths thin membranaceous; retinacula broad-ovate, slightly narrowed above, separated from one another by 1.5–3 times their diameter.

South Pacific (Australia).

A species approaching in size the smaller forms and states of *Z. marina* and more robust than any other of the *Zosterella* section.

Sterile forms referred to no. 5

Several New Zealand forms, resembling *Z. capricorni*, but with somewhat more oblong leaf tips and with 3 primary nerves, the two lateral often closely approximating the margins, and with more papery sheaths, seem probably distinct; but, in the absence of inflorescences, they are not, as yet, definitely to be placed.

6. *Zostera japonica* Aschers et Graeb

Leaf with apex obliquely rounded, slightly rounded emarginate (desquamate), primary nerves 3, the lateral near the margins, interstitial nerves 3–4; retinacula broadly lanceolate, obtuse, distant (about 6 times their diameter) from one another; testa smooth (never costate or "sulcate"). A narrow-leaved (1.5 mm. diam.) plant with elongated, erect fertile shoots and broadly lanceolate retinacula.

North Pacific (Japan, Saghalin).

B. Fertile Shoots Normally Short

7. *Zostera nana* Roth

Leaf with apex more or less unequal on the retuse (centrally desquamate) tip, primary

nerves 3, interstitial nerves 3 (1–4); retinacula narrowly oblong lanceolate, very remote (10–20 times their width) from one another.

North Atlantic (Europe).

8. *Zostera capensis* sp. nov.

Herba pumila (aut elatior) rhizomatibus repentibus gracilibus, plus minusve elongatis, internodiis lente compressis, fasciculis vascularibus lateralibus in latere utroque singulis, et fasciculis fibrosis in zonis corticis exterioris profunde dispositis; foliis curtis (8–15 cm. longis), angustis (0.75–1 mm. latis), nervis primariis 3, interstitiis 4 (2–5), apice anguste usque ad late, plus minusve inaequaliter rotundatis, per desquamiatione fisso emarginatis, vaginis lente apertis, tenui-membranaceis, ligulis curtis, delicatis; caulibus erectis fertilibus curtis 5–9 cm. altis, internodiis circa 3–4 (–7); spathis quam pedunculis 2-plo latioribus; spadicibus 1–3 mm. latis, 1–1.5 cm. longis, circa 7 floriis, retinaculis ovato-lanceolatis, plerumque 1–1.5 mm. longis, 0.5–1 mm. latis, remotis (circa 4-plo diam. proprio); seminibus circa 2 mm. longis, 1 mm. latis, late cylindricis, finibus utrisque late rotundatis, siccis distincta circa 20 striatis, testis humidis obscure longitudine 20 striatulis. *Z. nana* Auctt. var. ut planta Africae capensis designata.

Spec. Typ. Herb. Univ. Calif. no. 501,307. "Knysna Lagoon, Knysna, South Africa," Miss A. V. Duthie (Sc.D.), Jan., 1933.

Related to *Z. nana* Roth vegetatively, it differs decidedly in the broader retinacula and more striate testa, while from *Z. Muelleri* Irm. (*vera!*) of Australia it differs in its retuse (rather than broadly emarginate) leaf tip, in its usually smaller number of interstitial nerves, and in the smaller retinacula more attenuate above.

f. *elatior* forma nov. a forma typica per habitus robustiorem, foliis usque ad 45 cm. longis, 2–2.5 mm. latis, et caulibus erectis usque ad 15–18 cm. longis, internodiis circa 7, valde elongatis; floribus fructibusque ignotis.

Spec. typ., Herb. Univ. Calif., no. 501,046, "Knysna Lagoon, near Belvidere Heads, Knysna, South Africa," legit Miss A. V. Duthie (Sc.D.), no. 1212, Jan. 19, 1933.

Unfortunately neither of two specimens of this more robust form sent by Dr. Duthie were sufficiently advanced in anthesis to show either retinacula or seed characters. They seem to be forms of deeper water, while the typical form inhabits more shallow water.

9. *Zostera Muelleri* Irmisch.

Leaves with rounded tips unequally split by a deep triangular but fairly broad notch, primary nerves 3, interstitial nerves 4–5–6, sheath thin membranaceous, papery when dry, retinacula subquadrate, bluntly rounded at the apex, distant from one another by about 3 of their own diameters.

South Pacific (Australia (Victoria)).

It seems that both Ascherson and F. von Mueller, while having in mind a certain plant closely resembling *Z. nana* (and *Z. capensis*), confused with it certain sterile specimens more closely related to *Z. tasmanica*. What appears to be the type, presumably examined and reported upon by Irmisch, is preserved under no. 7 in the National Herbarium of Victoria, accompanied by notes and drawings of the retinacula. The internodes of the rhizome have only a single lateral bundle in each flank, not 2–5 as Sauvageau (*Jour. de bot.*, **5**, 64, 68, 1891) has indicated, nor are the fibre bundles in the outermost layers of the outer cortex reaching even to the epidermis as he has given (loc. cit.). Sauvageau was evidently misled by the confusion that has existed between the true *Z. Muelleri* and certain forms of the *tasmanica* group. No fibre strands have been noted in true *Z. Muelleri* around the central vascular bundles of the internodes (Sauvageau, loc. cit., 64, 68).

10. *Zostera novazelandica* sp. nov.

Herba pumila, rhizomatibus curtis, ascendentibus, gracilibus, 1–1.5 mm. diam., lente compressis, parce ramosis, fasciculis vascularibus in latere utroque singulis, fasciculis fibrosis in zonis corticis exterioris profunde dispositis; foliis fasciculatis, curtis 5–7 cm. longis, 1–1.5 mm. latis, nervis primariis 3 (2 marginalibus), nervis interstitiis 5, apice oblique truncata, late emarginata, denticulata, vaginis apertis, tenui-membranaceo-papyraceis, aetate provecta lacerato-fibrosis, ligulis moderate longis; caulibus erectis curtis, pedunculis circa 2 cm. longis, 1 mm. latis; spathis propriis circa 2 cm. longis, 3–4 mm. latis, moderate inflatis, laminis terminantibus 3 cm. longis, post anthesim cito deciduis; spadicibus 5 (–7) floriis, retinaculis late quadrato ovatis, 2 mm. altis latisque, in dimidiam supernam plus minusve costatis, remotis (1.5–2-plo diam.); seminibus elliptico oblongis, 3 mm. longis, 1.5 mm. crassis, siccis distincte, sed humidis obscure in longitudinem striatis, testis non costatis.

South Pacific (New Zealand, type specimen Bluff, N. Z., W. A. Setchell, Mar. 11, 1927, Herb. Univ. Calif., no. 313,091).

A low tufted species (as to type!), with scarious, fibrose, thin papery sheaths, leaves with broad, denticulate, shallowly but broadly emarginate apices, and broad, partially costate retinacula. New Zealand specimens (particularly from Port Nicholson, collected by T. Kirk, nos. 403 and 1491) referred earlier to *Z. Muelleri*, but later with doubt (cf. Ascherson and Graebner, loc. cit., 32).

A taller New Zealand plant (f. elatior ?), thus far seen only in sterile specimens, has been placed, at times, under *Z. capricorni*, and may be a distinct species, but the low New Zealand plants referred to *Z. Muelleri* probably all belong here.

Section 3. *Heterozostera* sect. nov.

A sectionibus Alegae et Zosterellae per structuram internodiorum rhizomatum (et caulium erectorum) abludens. Internodia 2–5 fasciculos vasculares in utroque latere ostendentia et fasciculis fibrosis in extissima complexu cellularum usque ad epidermidem positis. Spathae (adhuc notae) valde inflatae. Retinacula bene evoluta.

It seems best to separate *Zostera tasmanica* and its allies and place them under an independent section of their own, because of the multiple lateral vascular bundles of the internodes and because of the conspicuously inflated spathes. The members of the section differ from those of the *Alega* section in the number of lateral vascular bundles in the internodes, the possession of retinacula, the open sheaths and the inflated spathes, but resemble them in the external position of the cauline fibre bundles. They resemble the member of the *Zosterella* section in all characters except the duplication of lateral vascular bundles, the much more inflated spathes, and the external situation of the fibre bundles in the internodes.

11. *Zostera tasmanica* G. v. Martens

Leaves with apex rounded, denticulate and broadly but deeply indented, primary nerves 3 (2 marginal), interstitial nerves 5 (2–9); spathes broadly inflated; retinacula long, linear-lanceolate (3 mm. long by 0.75 mm. broad), distant from one another.

South Pacific (So. Australia, Tasmania ?).

A search was instituted among the Ascherson specimens in Herb. Berlin to elucidate the question of type, but nothing immediately connected with G. v. Martens was found. The type as described by Ascherson in 1867 (*Sitzungsber. d. Gesell. naturf. Freunde d. Berlin*, 15, and *Linnaea*, **35**, 168) seems to be a certain specimen received from Baron F. von Mueller, collected at Port Phillip. There is no such specimen in the herbarium at Berlin collected in or before 1867. There can be, however, little question of nos. 14 and 16 of Herb. Berlin belonging to *Z. tasmanica*. Of the truly Tasmanian specimens in

different herbaria from Tasmania, all are sterile and have rigid, black, erect, non-rooting stems. Such is the classical, but sterile, specimen, collected by Gunn, no. 1348, described as forming extensive beds at Georgetown, and quoted by J. D. Hooker (*Fl. Tasm.*, **2**, 44, 1860?) under "*Zostera marina?*," but placed by Ascherson (*Linnaea*, **35**, 168, 1867) under *Z. Muelleri*, from which latter species it differs fundamentally both in habit and in rhizome structure. It and other similar specimens may be ecophenes of *Z. tasmanica*, but there will be doubt until fertile specimens are collected.

Very similar is the single sterile specimen of *Zostera* known from Chile (Herb. Berlin, no. 13!), mentioned by Ascherson (loc. cit.) under *Z. Muelleri*. This is certainly more closely allied to *Z. tasmanica* than to any other species. It differs slightly from *Z. tasmanica*, but is very close to the Gunn specimen from Tasmania.

This preliminary survey owes much to the directors and curators of the following institutions, whose kindness in allowing examination of *Zostera* collections is most highly appreciated: Botanischer Garten und Botanisches Museum at Berlin, Royal Botanic Gardens at Kew, Botanisches Museum der Universität Zürich, National Herbarium of Victoria, Auckland Institute and Museum, Gray Herbarium, Herbarium of Yale University, New York Botanical Garden, Philadelphia Academy of Sciences and U. S. National Herbarium. For special foreign collections acknowledgments are gratefully made to Professor Dr. Carl Skottsberg of Göteborg, Sweden; Jean Feldmann of Paris, France; A. H. S. Lucas of Sydney, Australia; Lucy M. Cranwell of Auckland, N. Z.; Dr. (Miss) A. V. Duthie and (Miss) Edith L. Stephens of Capetown, South Africa; Shigeru Miki, Kyoto, Japan; Professor B. Hayata, Tokyo, Japan; Dr. K. Okamura, Tokyo, Japan; and Professor Y. Yamada and Masaji Nagai of Sapporo, Japan. From the United States and from Canada many correspondents have assisted (see Setchell, loc. cit., 391, 392).

A provisional key for the species based on vegetative characters is appended for assistance toward determining sterile specimens.

1.	Interstitial fibre bundles in outermost cortical layers of the internodes (even extending to the epidermis)	2	
1.	Interstitial fibres in inner portions of outer cortex (*Zosterella*)	6	
2.	Lateral vascular bundles 1 in each flank of internode (*Alega*)	3	
2.	Lateral vascular bundles 2–5 in each flank of internode (*Heterozostera*)	11.	*Z. tasmanica*
3.	Squamulae intravaginales 4	1.	*Z. marina*
3.	Squamulae intravaginales 2	4	
4.	Leaf tip broad, acuminate	4.	*Z. caulescens*
4.	Leaf tip broad, notched	5	
5.	Leaf tip slightly retuse	3.	*Z. asiatica*
5.	Leaf tip broadly and concavely emarginate	2.	*Z. caespitosa*
6.	Intermediate foliar vascular bundle (or bundles) present	5.	*Z. capricorni*
6.	Intermediate foliar vascular bundle not present	7	
7.	Interstitial fibre bundles 2–4	8	
7.	Interstitial fibre bundles 4–7	9	
8.	Leaf tip broadly shallow emarginate	7.	*Z. nana*
8.	Leaf tip narrowly shallow emarginate	6.	*Z. japonica*
8.	Leaf tip narrowly deep notched	8.	*Z. capensis*
9.	Leaf tip obliquely truncate, shallow emarginate	10.	*Z. novazelandica*
9.	Leaf tip oblong-rounded, deep notched	9.	*Z. Muelleri*

REMARKS ON SUPERCONDUCTIVITY

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Communicated August 11, 1933

1. *Introduction.*—Great efforts have been expended on the electron theory of metals during the last thirty years. The classical theory was at fault because it considered the electrons as entirely free. The quantum theory partly remedied this fault. In the first place, through application of the exclusion principle, the interaction between the electrons was *schematically* taken into account. In the second place the effect of the remaining ion lattice was introduced by placing the electrons into a fixed triply periodic field of force, corresponding to the distribution of the positive metal ions over the lattice. The results of all these efforts are still far from satisfactory. In particular the phenomenon known as superconductivity has so far defied all explanation.

The ultimate failure of the various versions of the electron theory of metals is not entirely surprising. Indeed none of these theories embraces those effects which make a metal a metal. It seems clear that any theory of metals can be satisfactory only if it contains some fundamental premises which essentially account for the distinction between metals and nonmetals. Such a criterion was given by K. F. Herzfeld.[1] This criterion is related to the mutual interaction of all the free electrons with the remaining ion-lattice.

2. *On Coöperative Phenomena in Metals.*—According to Herzfeld the so-called Lorentz field causes a self-perpetuating polarization in any substance whose molar refraction satisfies a certain condition. In the case of a cubic crystal or any isotropic substance (liquid) this condition is

$$4\pi N\alpha/3 > 1. \qquad (1)$$

Here N is the number of atoms per cm.^{2}, and α is the polarizability of the atoms. We have assumed that there is only one kind of atoms involved. The self-perpetuating polarization causes the atoms to be stripped of one or more of their electrons. As a result the electrons tend to occupy regions which are near the centers of the interspaces between the remaining ions. At sufficiently low temperatures ions and electrons will arrange themselves in a lattice. The electron lattice must of course be thought of as a dynamical average, as a static lattice would be unstable. The contention of this paper is that *this* type of an electron lattice must be subjected to a quantum mechanical investigation if one wishes to obtain a satisfactory theory of metals. Furthermore it must be noticed that the interaction of the ion-lattice with the electron-lattice again results in a self-perpetuating polariza-

tion (slight additional displacement of the two lattices relative to each other). I have previously discussed this type of polarization[2] in the case of heteropolar lattices such as LiF, NaCl, etc. I have also described how this polarization may cause the formation of secondary structures.

The consideration of coöperative phenomena of the type described, in conjunction with the introduction of a secondary structure, furnishes rational explanations for three groups of phenomena which are incomprehensible on the theory of ideal crystallographic lattices.[3] These phenomena are:

(*a*) The existence of *structure sensitive* properties in crystals. In particular it is now possible to give a rational explanation of the peculiar mechanical properties of crystals, including the extraordinary effects of cold working[4] and the actions of impurities.

(*b*) The occurrence, in crystals, of *characteristic lengths* of the order of $D = 10^{-4}$ cm. in contradistinction to the ordinary lattice constants of the order of 10^{-8} cm. Such lengths of the order of 1μ play an important rôle in recent theories of the frequency dependence of ferromagnetism, of recrystallization and of the plasticity of crystals. They also seem to be indispensable for the understanding of surface phenomena of crystals.

(*c*) The existence, in crystals, of one or more extremely sharp *transition points*, such as the melting point, the transformation points between various crystallographic modifications, the pseudo-transformation points, the Curie points and the abrupt changes of the direction of magnetization.

In the following I shall try to show that superconductivity is a phenomenon which also possesses the characteristics (*a*), (*b*) and (*c*). Superconductivity, therefore, must be related to those coöperative phenomena and secondary structures which are characteristic for the metallic state.

3. *On Superconductivity.*—The following are some of the characteristics of superconductivity.

(*a*) Superconductivity is greatly affected by plastic deformation, by the introduction of small amounts of impurities and by the magnetic field. It is therefore a structure sensitive property.

(*b*) Superconductivity can be made to disappear if the frequency of the induced current is increased beyond a certain limit. This fact can be explained if one assumes the existence of a surface layer of approximate thickness 10^{-4} cm. which can never be superconductive.[5] This layer exhibits the ordinary high resistance, and the dependence on frequency of the total resistance in the superconductive state can be interpreted on the usual theory of the skin effect. If this interpretation is correct superconductivity depends on a characteristic length of the order of 10^{-4} cm.

(*c*) Superconductivity is subject to *sharp transitions* inasmuch as it disappears abruptly at a definite temperature T_s. It also can be destroyed by the application of a magnetic field of rather definite magnitude. The rela-

tions between resistance and temperature resp. magnetic field very often exhibit hysteresis effects. This again indicates that superconductivity is a structure sensitive phenomenon.

From this enumeration it becomes evident that the problem of the superconductivity in many important respects is analogous to the problem of the existence of a crystalline state of matter. Indeed both problems involve analogous sets of characteristics (*a*), (*b*) and (*c*). I have previously proposed that the problem of the crystalline state may find a solution in the consideration of certain coöperative phenomena and related secondary structures.[3] Assuming that this contention is correct we may expect to find on the same grounds a solution for the problem of the superconductivity.

Our picture therefore is approximately as follows: The self-perpetuating polarization, or some similar phenomenon, which describes the coöperative interaction of the electron-lattice and the ion-lattice in a metal is responsible for the crystallization of this metal. It can be shown that this interaction at a definite temperature T_p is abruptly destroyed (similar to ferromagnetism). On our views this temperature T_p must be identified with the temperature T_m at the melting point. Consequently the energy of polarization ϵ_p (which is of the order of 10^{-14} ergs to 10^{-13} ergs per atom) must approximately be equal to the heat of fusion per atom. However, not all of the electrons in the metal are to be regarded as permanent members of the electron lattice. The thermal electrons (tail of the Fermi distribution) form an electron gas which is in thermal equilibrium with the electron lattice. For this electron gas there now exists another *second* chance to crystallize. Indeed, because of the formation of a secondary structure, the energy is not the same for all of the atoms in the crystal. In the "secondary" planes this energy will have some slightly different value, ϵ_p' such that

$$|\epsilon_p - \epsilon_p'| \ll |\epsilon_p|. \qquad (2)$$

The crystallization of a part of the thermal electrons in an arrangement which is predetermined by the secondary structure will take place at a temperature which is approximately given by

$$T_s = T_m |(1 - \epsilon_p'/\epsilon_p)| \ll T_m. \qquad (3)$$

If we identify T_s with the transition temperature of superconductivity we obtain at once an explanation of why this temperature is of the order of only 1° Kelvin. Why our secondary electron lattice may move through the crystal with practically zero resistance must be made the subject of a quantitative investigation. I mention here only that the ripples of the field of force (or the potential) along the secondary planes are very much flattened out as compared to the field of force throughout the more ideal

parts of the crystal. I have previously made use of this fact to explain the low mechanical strength of crystals against gliding (the word low is to be understood in comparison with high torsional modulii). Low mechanical gliding strength and superconductivity in our picture are closely related phenomena. It might be possible to obtain a check on this far reaching conclusion by closely investigating the gliding strength just below and above the transition point of a superconductor.

The above considerations do not claim to give more than a suggestion for a new theoretical basis on which the so far completely obscure superconductivity might be understood. A few more details may be mentioned which seem to furnish more support for our considerations. In the first place the characteristics (*a*), (*b*) and (*c*) find a rational explanation.

In the second place the difficulties related to the effects of grain boundaries in polycrystalline aggregates become less grave. These grain boundaries represent potential thresholds which would make superconduction impossible if this conduction were caused by the optical emptiness of ideal crystal lattices at low temperatures. The secondary structure, however, being the result of certain coöperative phenomena, can be expected to establish relations between neighboring microcrystals, even in the case that considerable amounts of impurities are present in the grain boundaries. The fact that such relations exist is suggested independently by some peculiar characteristics of the process of recrystallization and the resulting shapes of the grain boundaries.

In the third place the following important experiment can be understood. In a superconducting lead sphere a current around a certain diameter is induced. If the lead sphere is rotated around another diameter the current is partly carried along, that is, its axis of symmetry is turned. This fact would be incomprehensible if superconductivity were a volume phenomenon.

In the fourth place it is very important that the action of a magnetic field in reëstablishing a high resistance in a superconductor seems to be due to an action of the field on some predetermined arrangement of the elementary particles in the crystal. For a detailed account of this conclusion the original paper of de Haas should be consulted.[6]

4. *On Characteristic Lengths in Crystals.*—In the above a characteristic length D of the order of 10^{-4} cm. has been mentioned. With regard to its possible character I shall very briefly discuss a few characteristic lengths which can be constructed by combining some of the fundamental physical constants. A length of nuclear dimension is

$$\delta = e^2/mc^2 = 2.8 \times 10^{-13} \text{ cm}. \qquad (4)$$

For mutual interactions with other particles, at relative distances δ, the electrons probably entirely lose their individualities;[7] δ therefore also may

be regarded as a lower limit for the applicability of the exclusion principle.

A length of atomic dimensions is

$$d = h^2/me^2 = 20.8 \times 10^{-8} \text{ cm.} \qquad (5)$$

(The first hydrogen Bohr orbit has a radius $h^2/4\pi^2me^2$.) This length d may also be taken as the prototype of a crystal lattice constant.

Making use of the dimensionless fine structure constant $\alpha = 2\pi e^2/hc$ two more significant lengths may be constructed,

$$D_1 = d/\alpha = 137d$$

and

$$D_2 = d(M/m)^{1/3}/\alpha \qquad (M = \text{mass of an atom}). \qquad (6)$$

It can be shown that these lengths characterize the greatest region in which coöperative phenomena of the type of a self-perpetuating polarization can possibly establish uniform conditions. This limitation with regard to the radius of action is due to the finiteness of the speed of all physical signals.

The length D_1 also limits the size of a region in a crystal to whose electrons the exclusion principle in its usual form may sensibly be applied.

As the self-perpetuating polarization is intimately related to the highest frequencies of a crystal lattice, the lengths D_1 and D_2 may play a rôle in the determination of the lattice constants of secondary structures.

Still other characteristic linear dimensions can be constructed if one introduces great dimensionless numbers which necessarily occur wherever cooperative phenomena play a rôle. I have shown in another place[2] that d/Δ may be significant, where Δ is the relative electrostriction or magnetostriction resulting from a permanent electric resp. magnetic polarization of a crystal.

So far the exact theoretical determination of the secondary lattice constants of crystals has not been possible. The difficulty of finding a solution of this problem becomes evident if one considers the following inequalities,

$$|\epsilon_i| \gg |\epsilon_p| \gg |\epsilon_p - \epsilon_p'| \qquad (7)$$

where ϵ_i is the total energy of binding of a given particle in the crystalline state. The determination of the secondary lattice constant depends on the variations of the quantity $(\epsilon_p - \epsilon_p')$ as one varies the dimensions of the secondary structure. As these variations are many orders of magnitude smaller than ϵ_i or even ϵ_p, the difficulties are apparent. Fortunately, however, there are many other means of studying theoretically the actions of coöperative phenomena in crystals, some of which I have tried to discuss in this paper.

[1] K. F. Herzfeld, *Phys. Rev.*, **29**, 701 (1926).

[2] F. Zwicky, *Ibid.*, **38**, 1772 (1931).

[3] F. Zwicky, *Ibid.*, **43**, 270 (1933).

[4] F. Zwicky, *The Mechanical Engineer*, July issue (1933).

[5] R. Saenger, appearing in the *Phys. Review*.

[6] W. J. de Haas, *Jour. Physique*, **9**, 265 (1928).

[7] See F. Zwicky, *Phys. Rev.*, **43**, 1031 (1933), where I have given some experimental evidence for the loss of the individuality of electrons.

CONCENTRATION AND PURIFICATION OF A GROWTH INHIBITOR EXTRACTED FROM KIDNEY. A PRELIMINARY REPORT*

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Communicated August 10, 1933

In a previous paper[1] we described methods for extracting from rat kidneys a substance that inhibited proliferation of renal epithelium. The inhibiting agent was present in acid-alcohol and acid-aqueous extracts of both fresh and desiccated kidney. In the work reported in the present paper there is described the concentration and purification of the inhibitor.

Chemical Methods.—Fresh beef kidneys from older animals were quickly chilled and kept cold until delivered at the laboratory, where they were stripped of capsules and pelvic fibrous and fat tissues. The renal tissue was then passed twice through a meat chopper and at once covered with 0.2 per cent solution of hydrochloric acid in 60 per cent alcohol in the ratio of 500 cc. to 100 gm. of kidney. All of the acid-alcohol was not added at first and the pulp was ground to a finer consistency in a mortar before the entire quantity of acid-alcohol was added. After standing 24 hours at room temperature the acid-alcohol extract was freed of coarse undissolved kidney particles by pouring through cheese-cloth. In the first preparation made from beef kidney this separation was obtained by centrifugation. The volume of filtrate or supernatant alcoholic liquid was then reduced to about 1/30 by fanning. After driving off the alcohol the aqueous residue was brought to the original volume by adding water. This acid extract was neutralized with $N/1$ NaOH using Congo red as an indicator. The neutralization was stopped when a pH of 4.4 to 4.7 was reached. This was judged not only by the color change but also by the character of the precipitate formed. It is essential that the pH be kept below 5. Again if the liquid be too acid it does not clarify perfectly when the acetone is added. An equal volume of pure acetone was added, and the mixture placed in the ice-box overnight. The next day the aqueous acetone extract was filtered through filter paper. The pH of the clear filtrate was taken. It usually was within two points of that of the aqueous solution. The clear extract was fanned down to 1/30 volume to free it of acetone and then made up to the volume of the neutralized aqueous solution by addition of

water and trichloracetic acid. A solution of trichloracetic acid of about 20 per cent strength was added to make the final trichloracetic acid concentration 2.5 per cent. After standing overnight in the ice-box the precipitate was collected by decanting and centrifuging. The dense precipitate was fanned to dryness and transferred to a desiccator. After complete drying it was ground to a fine powder. It was then wrapped in ashless filter paper, placed in a Soxhlet extraction apparatus and extracted with chloroform until the lipids were removed. To make the product soluble each gram of the dry powder was dissolved in 30 cc. of 0.2 per cent hydrochloric acid in 60 per cent alcohol and 60 to 100 cc. of ether added to precipitate the dissolved extract. The sediment was washed free of alcohol with ether. The light grayish powder was then soluble in water. A large beef kidney yields 1–2 gm. of extract.

Biological Assay of the Extract.—The tests were made on young rats in the manner previously described by us. As a routine 2 or more litters of rats were used for the tests and the litter which showed the greater number of mitoses in the controls was selected. Averages below 10 mitoses per section are unsatisfactory.

The results are shown in table 1 where two typical litters are recorded. In the many tests made the maximum figure for an injected rat was almost always greater than the minimum number of mitoses found in any littermate control rat. Always the average of the injected rats was decisively below that of the controls.

TABLE 1

NO. OF RAT	WT. OF RAT IN GM.	AGE IN DAYS	MG. INJECTED	MITOSES PER SECTION
		FIRST LITTER		
534	35.0	23	75	5.0
535	34.5	23	50	10.6
536	32.0	23	25	7.3
537	34.5	23	none	33.3
538	44.5	23	none	38.0
539	34.5	23	none	25.0
		SECOND LITTER		
549	36.0	24	170	6.3
550	36.5	24	170	14.6
551	34.0	24	17	6.0
552	36.0	24	none	23.0
553	40.0	24	none	43.0

Summary.—A method is described for the concentration and purification from fresh beef kidneys of a substance that inhibits the mitotic activity of the renal epithelium of young rats.

* Aided by a grant from the NATIONAL RESEARCH COUNCIL.

[1] McJunkin, F. A., and Hartman, C. D., "Growth Inhibitor in Kidney Desiccates," *Amer. Jour. Pathology*. Accepted for publication May 5, 1932. In press.

THE ABOLISHMENT OF A DISCRIMINATION

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Communicated July 21, 1933

I.—The following formulation of the process of discrimination has been tested experimentally, as reported elsewhere.[1] Two stimuli, S_1 and S_2, eliciting the same response (R), possess some of their properties in common but differ also in some significant respect. In establishing a discrimination the reflex in response to one of them (S_1) is reinforced, while that in response to the other (S_2) is extinguished. Because of the similarity of the stimuli the two processes cannot go on independently, and their mutual interference constitutes the special problem of discrimination.

The resulting difference in behavior with respect to the two stimuli may be abolished in either of two ways. The reflex $S_1 - R$, hitherto reinforced, may also be extinguished, or the reflex $S_2 - R$, hitherto extinguished, may be reconditioned. The strengths of the two reflexes will then be equal, at some value near zero in the former case or near a maximum in the latter, and the discrimination will have disappeared. The present paper is concerned with the properties of these two kinds of change. One typical case of each sort is described. The conclusions are supported by about fifteen other cases, and no serious exceptions have appeared.

The responses of a white rat in pressing a small lever are recorded automatically[1] in the form shown in the figures, which are number-time graphs for the responses during one hour or more. Such a response may or may not be reinforced by the delivery of a pellet of food, at the will of the experimenter. The stimulation arising from the lever may be modified, for purposes of discrimination, by the addition of light from a small bulb.

Discriminations were established in the manner described elsewhere.[1] On the seventh day the strength of the extinguished members ($S_2 - R$) had reached the low values given by the slopes of the curves in figures 1*A* and 2*A*. The periodic reconditioning of the reinforced members ($S_1 - R$) occurred at the vertical bars above the records. The two cases differ in one respect: with Rat *KI* 46, S_1 consisted of the stimulation from the lever plus the light and S_2 of the stimulation from the lever only; with Rat *KI* 44 this was reversed. A comparison of the two methods is in preparation; the present argument is not affected.

II.—The first case, in which the previously reconditioned member is extinguished, is represented in figure 1. Record *B* was taken on the ex-

perimental day immediately following A, or on the eighth day of the discrimination. For about 45 minutes the usual periodic reconditioning of the response to the lever plus light and the intervening extinction of the response to the lever alone were carried out. After the eighth interval the light was turned on permanently and none of the subsequent responses was reinforced.

In making this change we establish the conditions for the extinction of $S_1 - R$, and we should therefore expect to obtain a curve which is similar to that previously reported for the extinction of a reflex of this sort.[2] The curve in figure 1 has the required properties. The only significant

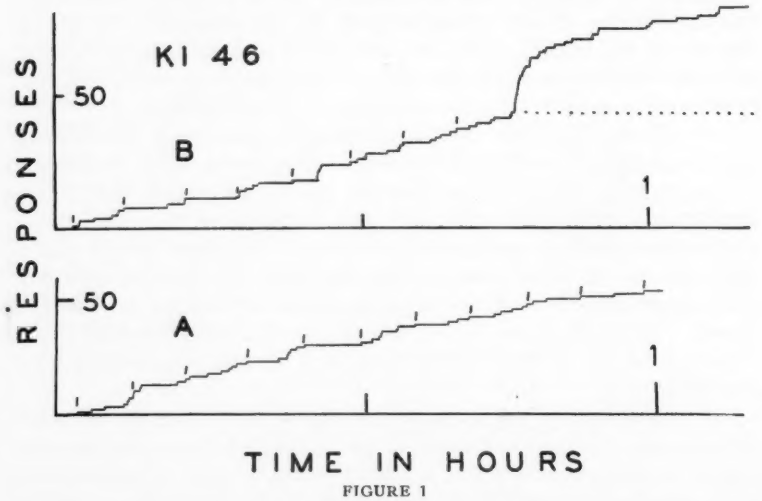

FIGURE 1

difference is the absence in the present case of a cyclic fluctuation. But this is to be predicted from the explanation advanced,[2] since the emotional effect to which the fluctuation is supposed to be due has here had ample opportunity to adapt out.

The area under the curve is somewhat less than that for original extinction and the curves are often smaller than the example here given. Apparently, then, the periodic reconditioning of the reflex $S_1 - R$ has not been wholly effective in maintaining its full strength or at least its full resistance to extinction.[3] It has already been shown that the concurrent reconditioning of $S_1 - R$ prevents the rapid extinction of $S_2 - R$, and we must now recognize a reciprocal effect of the extinction of $S_2 - R$ upon either the strength or the resistance to extinction of $S_1 - R$.

III.—In the second case, in which the previously extinguished member is reconditioned, the reconditioning could be effected very quickly by

successive reinforcements. But changes of this kind are too rapid to permit close analysis,[3] and for this and other reasons it is advisable to restore the extinguished reflex not to a maximal strength but to the intermediate strength observed under the original periodic conditioning prior

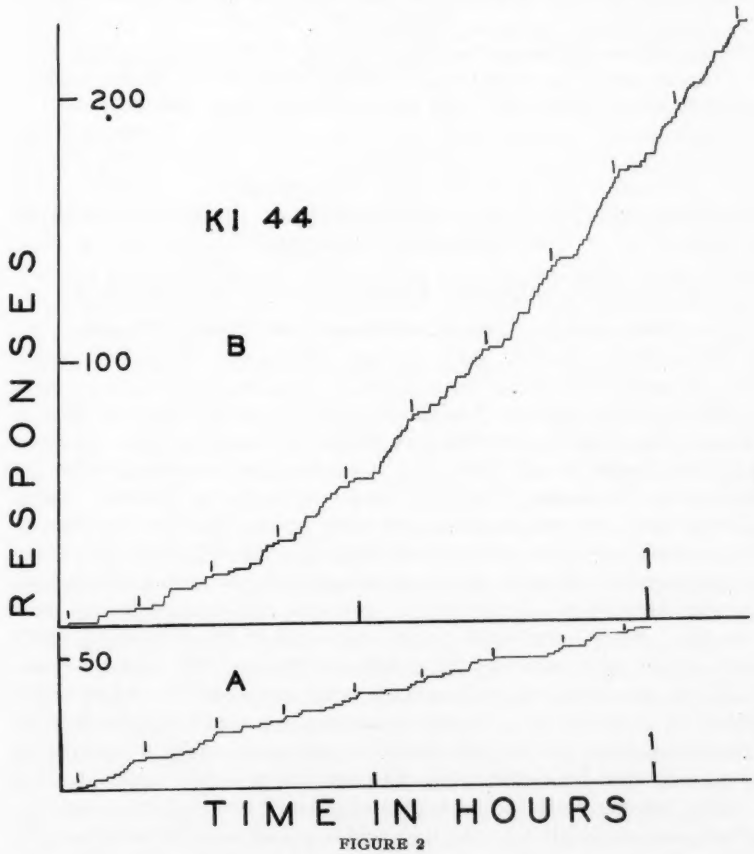

FIGURE 2

to any discrimination.[1] S_1 is therefore omitted altogether, and $S_2 - R$ is periodically reconditioned at the selected interval.

The typical result is shown in figure 2. Record B is for the day following record A. All responses are to S_2, and since these responses have not been reinforced during the discrimination, the rate begins at the low value given in record A. Periodically, however, the response is now reinforced, and the strength of the reflex gradually increases until it strikes a con-

stant value, which for our present degree of approximation is identical with that originally assumed prior to the discrimination. Except for minor local deviations the curve shows a smooth positive acceleration. It should be compared with figure 2 in the first reference,[1] which shows the original development of a constant rate under periodic reconditioning.

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CRYSTALLIZATION OF A COMPOUND OF HEMOGLOBIN AND CARBON DIOXIDE

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Communicated July 22, 1933

The question whether hemoglobin combines with CO_2 has been in dispute since such combination was claimed by Bohr[1] in 1909. Previous to Adair's[2] paper in 1925 there was little agreement concerning either the amount of CO_2 combined by Hb or the nature of the combination. Adair showed that the disagreement concerning the amount was attributable to previous failure to remove completely mineral base from the hemoglobin preparations used: in consequence saturation with CO_2 produced mineral bicarbonate in addition to any CO_2 combination formed with the Hb. Adair found that purified hemoglobin in solutions to which free carbonic acid was added did in fact combine reversibly with the latter, and that the compound in its osmotic behavior resembled a bicarbonate, $HbHCO_3$ or $Hb(HCO_3)_2$, the latter formula apparently representing the maximal amount of CO_2 that could be combined. Adair's experiments were performed with pure HbO_2 and pure CO_2 in water solution, so that the reaction was on the acid side of the isoelectric point of oxyhemoglobin, which is at about pH 6.7. At such acid reactions hemoglobin forms salts with acids in general; its behavior with carbonic acid was therefore what would be expected merely from the acid properties of H_2CO_3. In 1928, however, Henriques[3] reopened the question. He showed that several facts in the behavior of blood could be explained by assuming that hemoglobin, when at physiological pH, and therefore on the alkaline side of the isoelectric point, combines reversibly with CO_2, and by assuming that the "carbhemoglobin" so formed is not of the nature of a bicarbonate salt, but is rather a molecular combination with anhydrous CO_2, more nearly

analogous to the combination of Hb with O_2. The present state of the problem has been summarized by Stadie and O'Brien,[4] who offer evidence that carbhemoglobin does not exist, by Margaria,[5] who offers evidence that it does, and by Henriques,[6] who maintains his view, with some modification of the possible nature of the carbhemoglobin combination. In no case has such a compound been isolated.

In the present work we have succeeded in preparing oxyhemoglobin crystals containing carbon dioxide.

Rat blood was used, because of the ease with which its hemoglobin can be made to crystallize. The cells were washed several times by centrifugation with solutions containing $NaHCO_3$ in 30 to 60 millimolar concentration, NaCl sufficient to bring the total salt concentration to 160 millimolar, and as much free carbonic acid as the solution dissolved when saturated at 25° with air or oxygen containing CO_2 in tensions of from 60 to 670 millimeters. The pH of the solutions varied from 6.2 to 7.5, and therefore covered both sides of the isoelectric point of the oxyhemoglobin. Crystallization was begun by adding saponin to the centrifuged, washed cell mass. The mass was then stirred up with about an equal volume of the washing solution, from which crystallization continued for about 2 hours. Crystal mass and supernatant solution were separated and analyzed for CO_2 and water content, by weight. From the water content of the crystal mass the amount of adherent mother liquor and the CO_2 in the latter were estimated. Subtraction of the CO_2 of the adherent fluid from the total CO_2 of the crystal mass gave the CO_2 content of the solid crystals, which was found to vary parallel with the concentration of free carbonic acid in the mother liquor. The relationship was not entirely regular, but was approximately indicated by the formula, CO_2 bound per millimole Hb = $0.0015\ p_{CO_2}$, where p_{CO_2} represents in millimeters of mercury the tension of CO_2 in the gas with which the mother liquor was saturated, and a millimole of hemoglobin is the amount, about 16.6 grams, which combines with one millimole of O_2 or CO. When the CO_2 tension in the mother liquor reached about 670 mm. the hemoglobin crystals which separated contained as much CO_2 as O_2. Crystals forming at physiological CO_2 tensions contain only $^1/_{15}$ or $^1/_{20}$ as much CO_2.

Whether the CO_2 in the crystals is in a form other than bicarbonate of hemoglobin is a question which is under study.

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INTERCHANGE BETWEEN X- AND Y-CHROMOSOMES IN ATTACHED X FEMALES OF DROSOPHILA MELANOGASTER

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Communicated August 10, 1933

Introduction.—Attached X-chromosomes ($\widehat{XX}$) of *Drosophila melanogaster* separate occasionally (L. V. Morgan,[1] Anderson,[2] Muller and Dippel,[3] Dobzhansky,[4] Sturtevant[5]). The present cytological study was undertaken to compare the structure of detached X's with that of normal X-chromosomes.†

Seven separate detachments, occurring in cultures reared at about 25°C., were studied cytologically. Detachments *a*, *b*, *e*, *f* and *g* were detected as exceptional male progeny of $\widehat{XX}Y$ females. Such exceptional males, carrying the detached X's, were mated to attached X females. From the male larvae of these crosses, ganglion cells were selected for cytological study. The technique employed was a modified aceto-carmine method (Kaufmann[6]). Detachments *c* and *d* appeared in exceptional females, which were mated to wild-type flies. Ganglion cells of both male and female larvae from these crosses were investigated, since half of the members of each sex should carry the detached chromosomes.

Structure of the Sex Chromosomes.—The sex chromosomes of *D. melanogaster* during late prophases and metaphases of the somatic mitoses may be recognized by size and shape, and by the location of primary and secondary constrictions. A normal (unattached) X-chromosome (Fig. 9) shows an almost terminal primary constriction, or region of spindle fibre attachment. The most pronounced secondary constriction is slightly less than one-third of the distance along the chromosome from the proximal end. At this constriction in the X, one of the nucleoli develops (there are normally two per nucleus, although they may fuse to form one), so that during early prophases the X-chromosomes may be seen separated into two parts, usually by a distance equal to the diameter of the nucleolus (Kaufmann[6]). Another, less conspicuous secondary constriction exists about one-third of the distance from the distal end of the X. Knob-like portions often terminate the distal section.

The Y-chromosome at metaphase is a J-shaped body with two unequal arms separated by a primary constriction (Figs. 1, 4, 6, etc.). The longer arm shows a secondary constriction slightly distal to the middle. The shorter arm has a secondary constriction between a knob-like terminal region and a smaller, more or less spherical portion. A nucleolus develops

at this position in the Y and separates the short arm into two parts. This condition is clearly indicated during early prophases (Figs. 2, 5, 17, 18).

Attached X-chromosomes are held together at the primary constriction region (Fig. 21). During the prophases of the somatic mitoses the proximal attached ends are separated from the distal portions of the chromosomes by the nucleolus (Fig. 20).

All of the chromosomes are split longitudinally throughout the prophases and metaphases, although closely appressed or overlapping halves may obscure this detail.

Structure of Detached X-Chromosomes.—The seven detached X-chromosomes studied are not rod-shaped, but are either V-shaped or J-shaped. The V-type, represented by detachments *b, d, e, f*, is unequally armed, with one arm about three-fifths the length of the other (Figs. 10–18, chromosome $\widehat{XY^L}$). The J-shaped X-chromosome, represented by detachments *a, c, g*, consists of a long arm, comparable in length to the long arm of the V-shaped detached X. The short arm of the J, however, is only about one-third of the length of the long arm (Figs. 1–9, chromosome $\widehat{XY^S}$). V-shaped and J-shaped detachments seem to occur with about equal frequency, since four of the former and three of the latter type were encountered in the seven cases studied.

Comparison of detached X-chromosomes with the other chromosomes of the complement, and with unaltered X-chromosomes (Fig. 9), suggests that the longer arm of the V- or J-shaped structure is comparable in length to a normal X-chromosome. These detachments may be interpreted, therefore, as composed of a rod-shaped X-chromosome plus an extra arm. The nature of this extra arm was determined cytologically by a study of chromosome morphology in the giant cells of the ganglia and somatic pairing in the smaller ganglion cells. From this analysis it follows that the shorter arm of the V-shaped X corresponds to the long arm of the Y-chromosome, and that the shorter arm of the J-shaped chromosome corresponds to the short arm of the Y-chromosome.

The shorter arm of a V-shaped detached X often shows a nearly median secondary constriction. Such a constriction exists at a similar position in the long arm of the Y-chromosome. Further evidence that this type of detached X carries the long arm of a Y-chromosome is offered by somatic pairing in the smaller cells of the ganglia. In these cells side by side association of homologous parts is usually more complete than in the larger cells. In such metaphase plates as those shown in figures 11 and 12, the second, third and fourth chromosomes are paired. Pairing between X and Y presumably is between homologous regions, and exists between the long arm of the Y and the shorter arm of the V-shaped X.

Genetical evidence that the V-shaped X carries the long arm of a Y-

chromosome was secured by testing for fertility in males having this chromosome and the short arm of a Y-chromosome. Stern[7,8] has shown that the distal half of the long arm of the Y-chromosome has a fertility factor, or factor complex, K_1, and that the short arm of the Y possesses a supplementary factor, or factor complex, K_2. A male lacking either K_1 or K_2 is sterile.

The X-chromosome of detachment *b* of the present paper presumably carries the long arm of the Y-chromosome. Males with this chromosome and a normal Y were mated to $\widehat{XX}Y''$ females. (The Y″ chromosome, discovered by Stern,[7] consists of the short arm and the proximal half of the long arm of the Y-chromosome.) Males from this cross have the detached X- and the Y″-chromosomes. Such males are fertile. Since the Y″-chromosome has only the K_2 factor, the K_1 factor was introduced with the detached X-chromosome. Therefore this chromosome carries the long arm of the Y, and is designated as the $\widehat{XY}^L$ chromosome.

The J-shaped X-chromosome presumably represents a rod-shaped X plus the short arm of a Y-chromosome. This compound is designated therefore as the $\widehat{XY}^S$-chromosome. The short arm frequently shows a smaller proximal portion and a larger distal part. A similar structure exists in the short arm of the Y-chromosome.

In ganglion cells of male larvae which have the $\widehat{XY}^S$-chromosome and a free Y, one of the nucleoli develops at the constriction in the short arm of the free Y. The other nucleolus apparently develops at the proximal secondary constriction in the long arm of the $\widehat{XY}^S$-chromosome (Figs. 2, 5). It could not be determined with certainty that the short arm of the $\widehat{XY}^S$-chromosome does not participate in this procedure, but in most prophase figures it is evident that there is no such wide separation as in the short arm of the Y. During late prophases and early metaphases, after the disappearance of the nucleoli as visibly staining bodies, the short arm of the Y may appear relatively long, because of the continued wide separation of the terminal satellite-like body from the proximal portion. This

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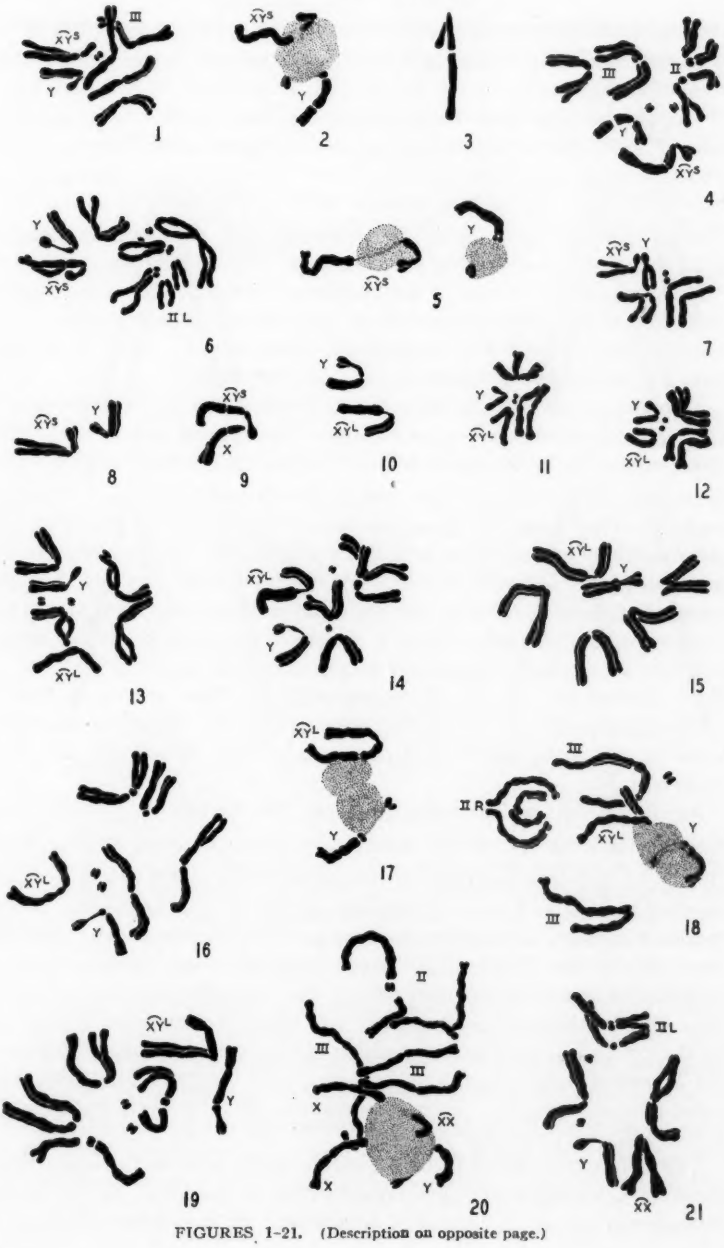
DESCRIPTION OF FIGURES 1–21

Figures 1–5, detachment *a;* 6–7, detachment *g;* 8–9, detachment *c;* 10–12, detachment *f;* 13, detachment *d;* 14–15, detachment *e;* 16–19, detachment *b.* Figures 20, 21 $\widehat{XX}Y$ ♀. Figures 9, 20, 21 from ganglia of females; all other figures from ganglia of male larvae.

Figures 2, 5, 17, 18, 20 prophases; figure 3, an $\widehat{XY}^S$ anaphase chromosome; figure 15, inception of anaphase; all other figures from metaphase plates.

Y = Y-chromosome; X = X-chromosome; $\widehat{XY}^S$ = detached X-chromosome bearing short arm of Y; $\widehat{XY}^L$ = detached X with long arm of Y; II, III = second and third chromosomes; II L = left (al) limb of second chromosome. Nucleoli stippled. X ca. 2700.



FIGURES 1–21. (Description on opposite page.)

continued extension explains why some figures show the short arm of the Y considerably longer than the homologous short arm of the $\widehat{XY}^S$-chromosome (Fig. 6).

Pairing occurs between these homologous arms in the smaller ganglion cells (Fig. 7). Especially interesting in this figure is the Y-chromosome, which is "backed into" the plate, and does not occupy the normal position, with the primary constriction in a more central position.

A fertility test with the $\widehat{XY}^S$-chromosome was not possible, since no stock was available with a free long arm of the Y-chromosome.

Exchange between X- and Y-Chromosomes.—The foregoing data suggest that detached X-chromosomes of *D. melanogaster* possess either a long arm or a short arm of a Y-chromosome attached to an entire X-chromosome. Two possible methods of origin are suggested.

The first is related to the separation of parts of X and Y by the nucleoli (Fig. 20) and assumes a type of nucleolar development and intra-chromosomal separation in the auxocytes, or in gonial cells, similar to that found in somatic cells. If both $\widehat{XX}$- and Y-chromosomes develop a common nucleolus, then upon the disappearance of that body, the proximal and distal parts of one of the attached X's might join together, whereas the proximal part of the other X might not rejoin its distal part and therefore remain free, or it might join with the distal section of the short arm of the Y-chromosome. In either case a J-shaped chromosome would result. Likewise a V-shaped chromosome might arise from union of that part of the Y having the spindle attachment with the distal section of that X which did not rejoin its proximal portion. Such a V-shaped chromosome, however, should be smaller and more nearly equal armed than the type observed.

An alternative explanation, current in this laboratory, postulates an exchange between $\widehat{XX}$ and Y during meiosis as the cause of detachment (Fig. 22). Either of two types of association of $\widehat{XX}$ and Y might give, as a result of a single exchange, a V-shaped and a J-shaped chromatid. Those resulting from an exchange between the proximal portion of the X and the short arm of the Y (Fig. 22*A*) would resemble those resulting from an exchange between the proximal part of the X and the long arm of the Y (Fig. 22*B*). Although neither of the two types of association is excluded by the cytological findings, the position of *bobbed* in the proximal region of the X, and of its allelomorph in the long arm of the Y, suggests that these regions are homologous. From this evidence alone synapsis would be expected as shown in figure 22*B*.

The assumption that detached X-chromosomes arise as the result of an exchange is strengthened by a consideration of the effect of certain environmental factors on the rate of detachment. The frequency in x-rayed

flies is higher than in the controls (Muller and Dippel,[3] Bridges and Gabritschevsky[9]). The latter authors, and Dobzhansky[4] found an increase of the rate of detachment with higher temperature. Dobzhansky also found an increased rate at low temperature. X-rays and high and low temperatures are known to increase frequency of crossing-over. There is

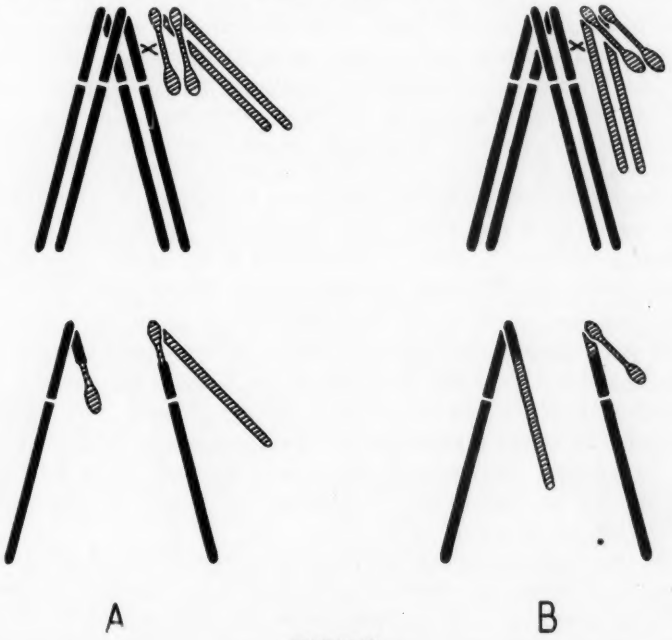


FIGURE 22

Diagram of two types of association of $\widehat{XX}$ and Y which would give, as a result of a single exchange, chromatids similar to the types of detached X's shown in figures 1–19. A—above, association between X and short arm of Y; below, the two chromatids resulting from the exchange (unaltered chromatids not figured). B—above, association between X and long arm of Y; below, chromatids resulting from the exchange. $\widehat{XX}$ in black; Y cross hatched.

of course the possibility that detachments resulting from x-rays are of a different type than those occurring in normal controls (Muller and Dippel).

The possibility of exchange between X and Y in the female was suggested by Stern[10] but was not verified. More recently Stern and Ogura[11] have demonstrated an exchange between $\widehat{XY'}$- and Y″-chromosomes of an $\widehat{XY'}$-Y″ male. This exchange occurred in the *bobbed* region of the two Y-chromosomes, and as a result a reconstructed Y-chromosome and a new combination ($\widehat{XY^s}$) were secured.

The $\widehat{XY}^{L}$ detachment of the present study is similar in structure to the $\widehat{XY}'$-chromosome discovered by Stern,[8,12] which arose during spermatogenesis of an XY individual, presumably through non-disjunction and attendant fusion of X and Y. This chromosome occurs with a frequency similar to that of the detached X's. Stern reported a frequency of the $\widehat{XY}'$ of one in 1500–2000 times. Anderson[2] reported a frequency of detachment of attached X's of about one in 1300. Schultz (unpublished) found a frequency of detachment of about one in 1100, in flies mated to x-rayed males, and about one in 1500 in the controls.

From this comparison, the suggestion of the present paper that exchange may occur between the inert regions of X- and Y-chromosomes, and from the knowledge that crossing-over may occur in the male (Stern and Ogura), it seems probable that Stern's $\widehat{XY}'$-chromosome may have arisen by an exchange between X- and Y-chromosomes in the male. The simplest assumption involves a single exchange between the proximal region of the X and the short arm of the Y. From this exchange an $\widehat{XY}'$ type of chromosome and a deficient chromosome, consisting of the spindle fibre region of the X, and a part of the short arm of the Y, could be obtained. Exchange between the long arm of the Y and the proximal region of the X could not be expected to furnish the $\widehat{XY}'$-chromosome, either on a basis of "homologous" pairing, or on the basis of inverted pairing with an inverted chiasma, suggested by Darlington[13] to explain this chromosomal aberration.

An inert region exists in the proximal portion of the X-chromosome (Painter,[14] Muller and Painter,[15] Dobzhansky,[16] Sivertzev-Dobzhansky and Dobzhansky[17]). The homology of the proximal portion of the long arm of the Y-chromosome with this region rests upon the location of the gene *bobbed* in the long arm. Probably the proximal regions of both long and short arms of the Y-chromosome represent "homologous" regions, either of which may pair with the inert region of the X-chromosome.

Since no exchange occurred between the *bobbed* region of the X and the "homologous" region of the long arm of the Y in Stern's test to detect crossing-over in that region in XXY females,[10] it seems probable that crossing-over between X and Y usually involves the short arm of the Y. This assumption is supported by a consideration of the probable origin of Stern's $\widehat{XY}'$-chromosome. It would also explain the origin of the types of detached X-chromosomes reported in the present paper.

Association of X- and Y-chromosomes in the first spermatocyte occurs over a short region only, in which the chromosomes assumedly are similar in construction (Metz[18]). Darlington[19] likewise found the sex-chromosomes of *D. pseudoōbscura* associated for a short region only. They were

held together by reciprocal chiasmata, either in the short arm or in the long arm of the Y. "In *D. melanogaster* the situation is probably comparable, except that the chiasmata can only be formed in the long arm." Whatever may be the situation in normal meiosis, the separation of attached X's and the compounding of X- and Y-chromosomes represent exceptional cases which may be explained by an exchange involving a single chiasma.

Detachments sometimes occur in groups of two or more from a single female. Sterling Emerson (unpublished) found one culture with 9 detachments. The grouping of detachments led Muller and Dippel[3] and Sturtevant[5] to suggest that detachment might occur in an oögonial division. This seems unlikely in light of the findings of the present paper, but cytological examination of a series of detachments of a single female should decide whether some other explanation than an exchange between $\widehat{XX}$ and Y is necessary.

Summary.—Detached X-chromosomes from $\widehat{XX}$Y females of *D. melanogaster* were observed to be of two types, either V-shaped or J-shaped. Both types seem to occur with about equal frequency. The former type is interpreted as a compound consisting of a rod-shaped X-chromosome and the long arm of the Y chromosome (the $\widehat{XY}^{L}$-chromosome). The J-shaped chromosome is regarded as a compound consisting of the X and the short arm of the Y (the $\widehat{XY}^{S}$-chromosome, a type of compounding of X and Y previously undescribed). The suggestion is offered that detachment occurs as a result of exchange between X- and Y-chromosomes. It could not be determined by cytological study of the detachments whether the exchange occurs between the long arm of the Y and the proximal region of the X, or between the short arm of the Y and the proximal region of the X. Other factors suggest that the short arm of the Y is usually involved.

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† I am indebted to Dr. Jack Schultz and to Dr. G. W. Beadle for supplying several of the detachments. I am further indebted to them and to Professors Th. Dobzhansky and Sterling Emerson for suggestions and criticism.

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POLLEN TUBE ESTABLISHMENT AND THE DEFICIENCY OF WAXY SEEDS IN CERTAIN MAIZE CROSSES[1]

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Communicated August 2, 1933

Investigations with the waxy gene of maize, described by Collins,[2] have been in agreement in that heterozygous plants (*Wx wx*) produced on an average a minus deviation from the expected 25 per cent of waxy seeds. Backcrosses of the type *wx wx* ♀ × *Wx wx*♂ also produced a significant deficiency of the recessive type, while from the reciprocal combination the expected 50 per cent of waxy seeds were obtained.

Brink and McGillivray,[3] Demerec[4] and Brink[5] have shown that heterozygous plants produce *Wx* and *wx* gametes in approximate equality in both micro- and megasporogenesis. Brink[6] has shown further that the deficiency of waxy seeds is more marked on plants of the constitution *su su Wx wx* than on *Su Su Wx wx* or *Su su Wx wx* plants. The additional deficiency associated with the homozygous *su su* plants was interpreted as a cytoplasmic effect.

Of the many explanations advanced for these observed deficiencies of waxy seeds, available genetic evidence has permitted of the discredence of all hypotheses except differential growth rates or differential development of gametophytes carrying the *Wx* and the *wx* genes. Since no consistent differences have been observed in the percentages of waxy and non-waxy seeds on the upper and lower portions of the segregating ears, there is no indication of a constant difference in growth rate. Brink and Burnham[7] recognize two stages in the development of the pollen tube. In the first stage the tube is dependent upon its own food reserves, while in the second stage it is dependent upon the food material in the style. They suggest that the differential effect of the waxy gene is exerted during the first stage. Brink[5] also suggests that a chemical basis for differential development of waxy and non-waxy gametophytes may exist only during this stage.

A method was devised to permit studying the germination of maize pollen and the establishment of the pollen tubes. Pollen is dusted on a fresh silk, allowing a few pollen grains to adhere to the stigmatic hairs. A portion of this silk is then fastened to a cover slip with vaseline, and the cover slip is inverted over a van Tieghem cell containing a drop of water to prevent desiccation. Germination can be readily observed under low power and the entrance of the pollen tube into the stigmatic

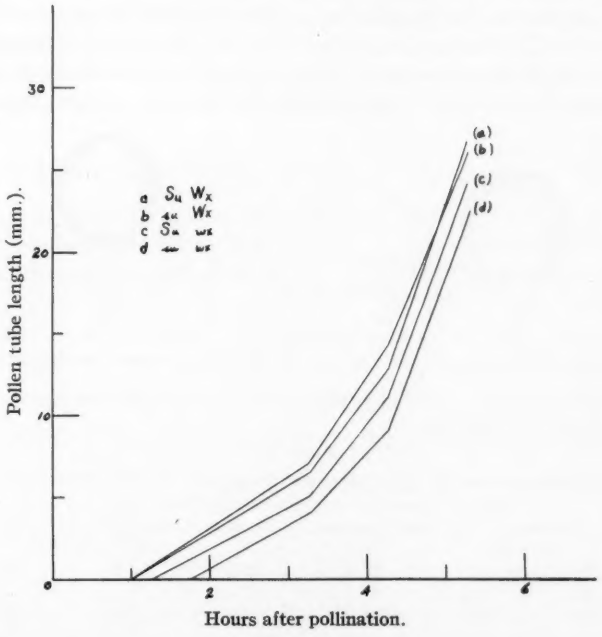

FIGURE 1

The growth rates, from the time of establishment, for pollen tubes of the four genotypes *Su Wx*, *Su wx*, *su Wx* and *su wx*.

hair and its growth to the silk proper can be followed under high power by means of the vigorous protoplasmic streaming in the pollen tube. Under favorable conditions germination of maize pollen is quite rapid. Established pollen tubes have been observed as quickly as five minutes after pollination. Under less favorable conditions germination may be delayed as much as an hour or more.

Data were obtained in this way on the relative rates of establishment of pollen tubes from closely related strains homozygous for the *Su Wx*, *Su wx*, *su Wx* and *su wx* genotypes. Paired comparisons were used to

eliminate as far as possible uncontrolled variables such as temperature, humidity at the time of pollen shedding, etc.

The time required for the germination of waxy pollen and the establishment of the pollen tube is consistently greater than for the non-waxy. When the *wx* pollen is produced by a *su su* plant, this difference is greatly increased. These differences apparently obtain regardless of the genotype of the silks used. This would have been expected on the basis of available genetic evidence. There is no significant difference in the time required for germination or establishment for pollen of the constitution *Su Wx* and *su Wx*. The differences in the mean time required for establishment of a series of paired comparisons and odds for the significance of the difference are presented in table 1. The times of establishment are not comparable

(*a*) (*b*)

FIGURE 2

Typical modes of attachment of *Wx* (*a*) and *wx* (*b*) pollen grains to the stigmatic hairs.

from row to row since they were determined under different conditions of temperature and humidity.

TABLE 1

THE MEAN DIFFERENCES IN TIME (MINUTES) REQUIRED FOR ESTABLISHMENT OF THE POLLEN TUBES OF THE INDICATED GENOTYPES

GENOTYPE		ESTABLISHMENT	TIME	DIFFERENCE	ODDS
a	*b*	*a*	*b*	*b* − *a*	
Su Wx	*Su wx*	28.4	32.2	3.8	832:1
Su Wx	*su Wx*	16.7	17.0	0.3	2:1
Su Wx	*su wx*	30.0	61.5	31.5	1999:1
su Wx	*Su wx*	19.8	27.2	7.4	475:1
Su wx	*su wx*	26.4	66.6	40.2	9999:1

Individual ears were pollinated with one of the four types of pollen *Su Wx*, *Su wx*, *su Wx* and *su wx*. The growth rate for each type of pollen tube from the time of establishment is shown in figure 1. The results indicate that there is no, or at most a very slight, difference in growth rates for the four types of gametophytes for the period studied. With a similar growth rate for the four types the slight deficiency of waxy seeds from *Su Su Wx wx* selfs and the greater deficiencies from *su su Wx wx* selfs are readily explainable on the basis of the longer time required for germination and establishment.

The time required for establishment appears to be directly related to the degree of contact between the pollen grain and the stigmatic hair or

silk. Non-waxy pollen grains become attached by a very broad base, which has its origin in the accumulation of moisture between the stigmatic hair and pollen. The contact force is often so great that the pollen grain is noticeably flattened. With waxy pollen this condition is much less striking and may be entirely lacking. A typical example of the attachment of germinating *Wx* and *wx* pollen grains is shown in figures 2*a* and *b*, respectively. The drawings were made from photographs taken 20 minutes after the pollen was placed upon the silks.

Differential pollen tube growth has been advanced in many cases as an explanation for the deficiency of a particular genotype. In view of the findings presented in this paper, it would appear desirable hereafter to distinguish clearly between differential establishment and differential growth.

[1] Contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry.

[2] Collins, G. N., *U. S. Dept. Agr. Bull.*, **161**, 1–30 (1909).

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THE EFFECT OF HOMOZYGOUS GENES FOR SELF-STERILITY

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Communicated July 24, 1933

East and Yarnell (1929)[1] have reported the isolation of fifteen allelomorphs for self-sterility in the species *Nicotiana Sanderæ* Hort., *Nicotiana alata* Lk. and Otto, and in hybrids between them. These allelomorphic genes differ from each other in the sense that any plant bearing a member of the series S_1 to S_{15}, is incompatible with pollen bearing the same member of the series, although it is compatible with pollen bearing any other member of the series. Thus an S_1S_2 plant exhibits incompatibility to pollen carrying allelomorphs S_1 or S_2, although it shows compatibility to pollen carrying allelomorphs S_3 to S_{15}. The incompatibility shown is a retardation of the pollen-tube growth rate sufficient to prevent fertilization from occurring under ordinary circumstances. There is no impediment to fertilization if the pollen tube reaches the micropyle.

It has been found that when incompatible matings are made on immature flowers—flowers which will not open until from 24 hours to 48

hours have elapsed—the retardation of pollen-tube growth characteristic of flowers pollinated at maturity is materially lessened. Under such circumstances fertilization will frequently occur. By this means plants homozygous for specific S allelomorphs can be obtained.

Utilizing this method, plants homozygous for each of the allelomorphs of the series have been produced (S_1S_1 to $S_{15}S_{15}$).

During the execution of this work, further evidence was obtained that the self-sterility genes involved are allelomorphs of the single gene S. Plants S_xS_y selfed yielded only plants S_xS_x, S_xS_y and S_yS_y; while plants S_xS_z selfed yielded only plants S_xS_x, S_xS_z and S_zS_z. In addition, matings involving the basic color gene C, which was shown to be linked with S_1, S_2 and S_3 by Brieger and Mangelsdorf (1926),[2] yielded corroborative results for five other allelomorphs.

Omitting, for the moment, two cases that will be discussed separately—certain families containing S_3S_3 and a family resulting from S_2S_5 selfed in the bud—the plants homozygous for these various allelomorphs were normal in appearance and in behavior. Different populations of seeds, when sown, showed great variation in germinating power; but there was no indication that this variation was correlated with the possession of a specific allelomorph. Similarly, there were marked differences in the percentages of good pollen as judged by examinations in aceto-carmine, lactic acid, etc., but these differences were not occasioned by the action of the sterility allelomorphs. Out of some hundreds of plants examined, only about 3 per cent had less than 85 per cent of apparently viable pollen. This small group had percentages of bad pollen up to 90, but the distribution of these more highly sterile plants was clearly unassociated with any specific S genes.

The S allelomorphs could be transferred to plants having a variety of genetic constitutions, as any other normal gene can be so transferred, without materially disturbing the specific behavior. This conclusion, perhaps, requires some explanation. *N. Sanderæ* and *N. alata* possess 9 pairs of chromosomes which undergo normal distribution in all combinations thus far examined. The stocks used in our study differed in at least 10 color genes, and in flower size genes which resulted in variations in corolla length from 25 mm. to 80 mm. Many families were examined which were segregating for not less than 6 of these genes. And there was no evidence of abnormal behavior of the S genes in combination with particular genetic constitutions. In other words, if the chromosome containing the S gene and the C gene be designated Chromosome 1, it makes no difference whether this chromosome contains C or c, or whether the remaining chromosomes carry particular combinations of color genes or size genes, the plant is still a normal plant and exhibits the compatibility relationships of the specific S gene involved.

The two cases that were originally thought to be exceptions to this conclusion are as follows.

S_3S_3 plants, as described by East and Mangelsdorf (1926),[3] are generally characterized by a kind of rachitic habit of growth, curled leaves, female sterility and partial male sterility (40 to 60 per cent). The seeds germinate poorly and the seedlings are weak. With care, however, the plants can be grown to maturity. Approximately 400 such plants have been raised from about 30 families. From this lot, 1 plant was found that had normal characteristics with the exception of female sterility; and the female sterility was not complete. When selfed, this plant, which was proved to be S_3S_3 by numerous tests, gave 3 plants also normal in appearance and having S_3S_3 behavior. It thus appears that the abnormalities ordinarily characterizing S_3S_3 plants are, with the possible exception of a tendency to female sterility, not the expression of the S_3S_3 gene, but the expression of another gene closely linked to S_3.

The population coming from the S_2S_5 plant selfed, yielded plants having abnormally narrow leaves. The first few data indicated that this trait was the effect of the S_5 gene in the homozygous condition. Further study, however, showed that the gene for narrow leaves is independent of the S genes. S_2S_2 plants and S_5S_5 plants were produced both with normal leaves and with narrow leaves.

In 1930, Castle[4] advanced the presumption that "a self-sterile organism is one which harbors a gene lethal in the process of fertilization and which is able to perpetuate its line only by avoiding self-fertilization or fertilization by gametes bearing the same lethal gene." He says: "We are now prepared to define a self-sterile plant. It is one which bears two different allelomorphs of the same gene essential to the growth of the pollen tube, *both being lethal*, that is, fatal if homozygous."

I find no support to this hypothesis. All the homozygous forms of the 15 allelomorphs isolated in *N. Sanderæ* and *N. alata* have been produced and grown to maturity. No allelomorph is lethal in the homozygous condition. The S allelomorphs behave as ordinary non-lethal mutations, though playing their own peculiar physiological rôle. Their reactions are, I believe, analogous to certain immunological reactions. The style bearing S_x reacts against the pollen tube bearing S_x in a specific manner, thus retarding its growth. Details of this process will be described in a forthcoming paper.

Castle's belief in the lethal nature of homozygous self-sterility genes, in advance of experimental evidence, presumably flows from the feeling that it is the logical consequence of his ideas on sexuality. Among these ideas, which are expressed with characteristic lucidity,[4] is the thought that it is an advantage of sexuality that any number of lethal genes may be present without harmful effect, provided none becomes homozygous

through inbreeding. In certain specific connotations, this idea is obviously true; and from it one might be led to inquire if genes producing cross-fertilization by physiological methods may not be lethal in the homozygous condition, though there is certainly no logical necessity for such a deduction. But, in a broader sense, it seems to me that the persistence of bisexuality and of various contrivances for promoting cross-fertilization among hermaphroditic organisms must have quite a different interpretation. Unless heterosis should later be proved to be in part independent of genic constitution, the sole advantage of the mechanisms promoting cross-fertilization lies in the opportunity afforded for the recombination of a given mutation with genic constitutions other than that in which the mutation appeared. The presence of the allelomorph of the mutation permits it to be carried on if deleterious or even lethal when homozygous. Ultimately, it may be carried into genic combinations (or into environments) where it proves to be beneficial. These important considerations Fisher, Wright and Haldane have developed with great care and rigorous logic. But it must not be thought that for these reasons the presence of a single allelomorph of any mutation whatever is no burden to the individual carrying it. In the work on maize, where this type of observation has been carried on most intensively, it is becoming increasingly evident that complete dominance rarely, if ever, occurs. A gene which is deleterious in the homozygous condition usually shows some effect of its nature when in the heterozygous condition. This is the chief reason why over 100,000 strains of maize are now being inbred in the United States preparatory to making various first-generation hybrid combinations for commercial maize-growing. The investigators are endeavoring to eliminate deleterious genes.

If, then, one is not to look for the origin of self-sterility as a result of the contention that self-sterility genes are lethal when homozygous, what is the most probable means of origin? I have called attention, in a previous paper, to the difficulty in accounting for the origin of a phenomenon so widespread among the flowering plants by the hypothesis of mutation from self-fertility to self-sterility. A mutation from S_f (self-fertility) to S_1 (self-sterility) could be carried along in the heterozygous condition, but no self-sterile plants could appear unless a second self-sterility allelomorph S_2 should appear. And no self-sterile race could arise until a third self-sterility allelomorph S_3 had appeared.

Recently, I have made an extended study of the rapidity of pollen-tube growth in the various homozygous self-sterility strains. It appears that the rate of pollen-tube growth at a given temperature is different in different genotypes. For example, the growth rate is very slow in S_4S_4, not quite so slow in S_1S_1, faster in S_2S_2 and much faster still in $S_{15}S_{15}$. Let us suppose that mutations to a number of allelomorphs have appeared at a

given locus in a self-fertile race of plants, each mutation tending to fix the growth of pollen tubes at a specific rate characteristic of that mutation in a constant environment. One need not suppose that the slowest rate of growth exhibited as the result of mutation will prevent the male gametes from entering the micropyle; but there will be competition and selective fertilization such as Jones has proved to occur in maize. Now let us assume that a single mutation occurs which permits the tissues of the style to form a substance that reacts specifically and antagonistically to the growth of a pollen tube carrying a gamete with a like factor for pollen-tube growth. The advantages of such a cross-fertilization system should presumably be such that a self-sterile race would shortly be established homozygous for the latest gene which one might call the immunity gene *i*. This race would have as many self-sterility allelomorphs, or *S* genes, as there had been mutations in the pollen-tube growth-controlling locus. One may be able to distinguish between these two hypotheses at some future time if races (or species that are fertile together) are discovered that differ by the genes *I* and *i*. At present no such races are known. I have worked with *N. Langsdorffii* Weinm., which is a self-fertile species having the same chromosome number as the self-sterile species *N. Sanderæ* and *N. alata*. *N. Langsdorffii* will cross freely with the other two species; and the chromosome distribution in the hybrids is normal. But *N. Langsdorffii* is a self-sterile species in the sense that it has the immunity reaction characteristic of *N. Sanderæ* or *N. alata* against the self-sterility allelomorphs $S_1 \ldots S_{15}$, if one may judge by the reactions of the hybrids. The hybrid *N. Langsdorffii* (S_fS_f) $\times$ *N. Sanderæ* (S_1S_1, etc.) reacts against pollen bearing S_1. Thus it seems, though the argument is not critical, since complete genoms from each of the two species are present, that presumably *N. Langsdorffii* is also *ii* in the sense that I have used the designation.

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[2] Brieger, F. G., and Mangelsdorf, A. J., these PROCEEDINGS, **12**, 248–255 (1926)
[3] East, E. M., and Mangelsdorf, A. J., *Genetics*, **11**, 466–481 (1926).
[4] Castle, W. E., *Amer. Naturalist*, **64**, 481–494 (1930).

ON CRITERIA FOR FOURIER CONSTANTS OF L INTEGRABLE FUNCTIONS

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Communicated July 21, 1933

In a note published in volume 18 (pp. 396–399) of these PROCEEDINGS I gave certain criteria which would assist in determining whether or not a set of constants a_n ($n = 0, 1, 2, \ldots$; $a_n \longrightarrow 0$) were the Fourier cosine coefficients of an L integrable function. The object of the present note is to obtain more general criteria for the case where the constants *are* Fourier cosine coefficients.

The criteria in question involve differences of non-integral or integral order formed from an infinite set of constants. We define these as follows for any set of constants $a_0, a_1, a_2, \ldots, a_n, \ldots$.

$$\Delta^{k+1}a_n = a_n - (k+1)a_{n+1} + \frac{(k+1)k}{1.2}a_{n+2} - \ldots \quad (k > 0). \quad (1)$$

For a non-integral value of k these differences are the limit as p becomes infinite, provided this limit exists, of the expressions

$$\Delta_p^{k+1}a_n = a_n - (k+1)a_{n+1} + \ldots + (-1)^{p-n}\frac{(k+1)k.\ \ (k-p+n+2)}{1.2.\ \ .(p-n)}a_p. \quad (2)$$

If for a series Σu_n, we set

$$s_\lambda = \sum_{n=0}^{n=\lambda} u_n; \quad S_n^{(k)} = \sum_{\lambda=0}^{\lambda=n} A_{n-\lambda}^{(k-1)} s_\lambda, \quad (3)$$

where

$$A_n^{(k-1)} = \frac{k(k+1)\ldots(k+n-1)}{n!} \ (n = 1, 2, \ldots), \ A_0^{(k-1)} = 1, \quad (4)$$

we have the identity

$$\sum_{n=0}^{n=p} u_n a_n = \sum_{n=0}^{n=p} S_n^{(k)}\ \Delta_p^{k+1}a_n \qquad (\Delta_p^{(k+1)}a_p = a_p),$$

by means of a transformation analogous to the well-known Abel transformation. For any series for which the $S_n^{(k)}$ remain bounded, for a $k > 0$ and all n, we may, if the a_n's satisfy the conditions

$$\lim_{n\to\infty} a_n = 0, \quad \sum_{n=0}^{\infty} n^k\left|\Delta^{k+1}a_n\right| < \infty \ (k > 0), \quad (5)$$

allow p to become infinite in the above identity and thus obtain the result

$$\lim_{p \to \infty} \sum_{n=0}^{n=p} S_n^{(k)} \Delta_p^{k+1} a_n = \sum_{n=0}^{\infty} S_n^{(k)} \Delta^{k+1} a_n = \sum_{n=0}^{\infty} u_n a_n,$$

the convergence of the series in the second and third members being assured[1] by the conditions on the $S_n^{(k)}$ and the a_n.

Our criteria may now be stated in the following theorem.

THEOREM. *If the set of constants* $a_0, a_1, a_2, \ldots, a_n, \ldots$ *satisfy the conditions* (5), *then the series*

$$\tfrac{1}{2}a_0 + a_1 \cos\theta + a_2 \cos 2\theta + \ldots + a_n \cos n\theta + \ldots \qquad (7)$$

will converge in the open interval $(0 < \theta \leqq \pi)$, *and will represent there an* L *integrable function whose Fourier cosine development is given by* (7).

This theorem follows readily from the preceding discussion and certain properties of the trigonometric series

$$\tfrac{1}{2} + \cos\theta + \cos 2\theta + \ldots + \cos n\theta + \ldots. \qquad (8)$$

If for the latter series we define $S_n^{(k)}(\theta)$ in the manner indicated by (3) and set

$$\sigma_n^{(k)}(\theta) = \frac{S_n^{(k)}(\theta)}{A_n^{(k)}}, \qquad (9)$$

so that the $\sigma_n^{(k)}(\theta)$ are the Cesàro means of order k for the series (8), we have[2]

$$\int_0^{\pi} |\sigma_n^{(k)}(\theta)|\, d\theta < K \quad (k > 0;\ n = 0, 1, 2, \ldots), \qquad (10)$$

where K is a positive constant. We may express this property by saying that for the series (8) the Cesàro means of order k are *bounded in the mean* for the interval $(0, \pi)$. Because of the conditions on the a's and the boundedness of the $S_n^{(k)}(\theta)$ for the series (8) in the interval $0 < \delta \leqq \theta \leqq \pi$), we may infer the convergence of the series (7) in that interval to a function

$$f(\theta) = \tfrac{1}{2}a_0 + a_1 \cos\theta + \ldots = \sum_{n=0}^{\infty} S_n^{(k)}(\theta) \Delta^{k+1} a_n. \qquad (11)$$

The series on the right-hand side of (11) converges absolutely and uniformly in the interval $0 < \delta \leqq \theta \leqq \pi$ and can therefore be integrated term by term in that interval. Thus we have

$$\int_{\delta}^{\pi} |f(\theta)|\, d\theta \leqq \sum_{n=0}^{\infty} \left(\int_{\delta}^{\pi} |\sigma_n^{(k)}(\theta)|\, d\theta \right) \frac{A_n^{(k)}}{n^k} n^k \left| \Delta^{k+1} a_n \right|. \qquad (12)$$

As δ approaches zero, the right-hand side of (12) approaches a definite limit in view of conditions (5) and the boundedness in the mean of the $\sigma_n^{(k)}(\theta)$ in the interval $(0, \pi)$. Thus the left-hand side of (12) does also, and the existence of the resulting integral shows that the function $f(\theta)$ is an L integrable function whose Fourier cosine development is (7).

The second condition in (5) involves a parameter k, and thus we have different criteria for different values of k. It can be shown that if a set of constants, a_n, satisfy this condition for a particular $k > 0$, they will also satisfy it for any smaller positive value of k. Thus the scope of our theorem increases as k decreases. For $k = 1$, we have, as a special case, a criterion previously given by Kolmogoroff,[3] which is somewhat more general than the criterion of our previous note. For values of k in the interval $0 < k < 1$, we have more general criteria than that of Kolmogoroff.

[1] S. Chapman, "On Non-Integral Orders of Summability of Series and Integrals," *Proc. Lond. Math. Soc.*, ser. 2, **9**, 369–409 (1911); see in particular § 15.

[2] L. c. § 20. Cf. also T. H. Gronwall, "On the Summability of Fourier's Series," *Bull. Amer. Math. Soc.*, **20**, 139–146 (1913).

[3] Kolmogoroff, "Sur l'ordre de grandeur des coefficients de la serie de Fourier-Lebesgue," *Bull. Acad. Polon.* (A) **1923**, 83–86 (1924). This paper came to my attention subsequent to the publication of my previous note.

GROUPS WHOSE OPERATORS HAVE NO MORE THAN THREE DISTINCT SQUARES

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Communicated July 31, 1933

A necessary and sufficient condition that every operator of a given group G has the same square is that G is an abelian group of order 2^m and of type $(1, 1, 1, \ldots)$. If the operators of G have two distinct squares it is of order 2^m since every operator of a group of odd order is a square of one and only one of its operators. Hence it results that the condition that the operators of a group have two and only two distinct squares is equivalent to the condition that the order of each of its operators divides 4 and that its operators of order 4 have a common square. The groups which satisfy these conditions have recently been determined[1] and hence we shall confine our attention in what follows to groups whose operators have three and only three distinct squares.

The order of such a group cannot be divisible by a prime number which exceeds 3 and when it is divisible by 3 the group contains a single subgroup of this order, whose operators are the squares of all of the operators of the group. The orders of these operators are therefore 1, 2, 3 or 6. If the group is non-abelian it contains an abelian subgroup of index 2 composed of all its operators which are commutative with its two operators of order 3. This abelian group is the direct product of the group of order 3 and the abelian group of order 2^m and of type $(1, 1, 1, \ldots)$. All the

remaining operators of G are of order 2 and hence the entire group is the direct product of the non-cyclic group of order 6 and the abelian group of order 2^m and of type (1, 1, 1, ...) whenever it is non-abelian. When it is abelian it is the direct product of the latter group and the group of order 3. In what follows we shall therefore confine our attention to the case when the order of G is of the form 2^m.

To prove that the two distinct squares of the operators of order 4 in G appear in its central it is only necessary to observe that such a square is transformed into itself by every operator of order 4 in G since such an operator transforms its own square into itself. It is also transformed into itself by every operator of order 2 in G since every two non-commutative operator of order 2 contained in G generate the octic group and hence transform at least one of these two squares into itself. Hence it results that these two squares generate the four group and that this appears in the central of G. As the corresponding quotient group involves only operators of order 2 this four group involves the commutator subgroup of G. That is, *if a group of order 2^m involves three and only three operators which are squares then these three operators generate the four group, appear in the central of the group, and generate its commutator subgroup.*

As G cannot be abelian its commutator subgroup is of order 2 or of order 4. We shall first consider the former case. It is known that there are two and only two such groups of order 16. Hence G must contain invariantly one of these two groups. Every co-set of G with respect to one of these invariant subgroups involves four operators which are in the central of G. These must be of order 2 since an operator of order 4 contained in G cannot be commutative with two such operators which have different squares. This proves the following theorem: *There are two and only two groups of order 2^m, $m > 3$, which separately involve three and only three operators which are squares and have a commutator subgroup of order 2. They are the direct products of the two distinct groups of order 16 which have these properties and the abelian group of order 2^{m-4} and of type* (1, 1, 1,...). It may be noted that exactly one-half of the operators of one of these groups are of order 4 while three-fourths of the operators of the other have this common order.

It remains to consider the case when the commutator subgroup of G is of order 4. We shall first prove that such a group involves invariantly one of two groups of order 32 which have in common the abelian group of order 16 and of type (2, 1, 1) but involve no other abelian subgroup of order 16. The conjugate operators of G are commutative since its commutator subgroup is in its central. Just as before we see that G contains invariantly at least one of the two groups of order 16 whose operators have three distinct squares and hence G involves operators which have four conjugates under G since it cannot be the direct product of such a

group of order 16 and the abelian group of order 2^{m-4} and of type (1, 1, 1, ...). In fact, such a subgroup of order 16 involves at least two sets of four conjugates under G. Hence this subgroup is extended under G by an operator which is commutative with all the operators of one of its abelian subgroups of order 8 but transforms this subgroup of order 16 according to an outer isomorphism.

The group of order 32 thus obtained contains the abelian subgroup of order 16 and of type (2, 1, 1) since this subgroup must involve operators of order 4. As each of the 16 operators of this group of order 32 which do not appear in this abelian subgroup has four conjugates thereunder it results that there is one and only one such group of order 32 when the extending operator is of order 2 and there is also one and only one such group when each of the remaining 16 operators is of order 4. Each of these two groups of order 32 involves both of the groups of order 16 which contain separately three operators which are squares. This proves the following theorem: *If a group of order 2^m which involves three and only three operators which are squares has a commutator subgroup of order 4 then it contains invariantly the abelian group of order 16 and of type (2, 1, 1) and also each of the two groups of order 16 which contain separately three and only three operators which are squares.*

It has now been proved that G must involve invariantly at least one of the two groups of order 32 which are characterized by the fact that they involve separately three and only three operators which are squares under the group and that four of their operators are conjugate thereunder whenever G has a commutator subgroup of order 4. If each of the remaining operators of G transforms the operators of this subgroup according to an operator in its group of inner isomorphisms then G is the direct product of this invariant subgroup of order 32 and the abelian group of order 2^{m-5} and of type (1, 1, 1, ...). There are therefore two and only two such groups of order 2^m, $m > 4$. In one of these the number of the operators of order 4 is one-half of the order of the group while in the other it is equal to three-fourths of this order. If we include the groups whose commutator subgroups are of order 2 there are therefore at least four groups of order 2^m, $m > 4$, which involve separately three and only three operators which are squares. In two of these the commutator subgroup is of order 2 while the order of this subgroup is 4 in the other.

If a group of order 2^m contains three and only three operators which are squares thereunder and involves a central whose order exceeds 4 then it is the direct product of such a group whose central is of order 4 and an abelian group of type (1, 1, 1, ...). This theorem results from the fact that each of the given groups of order 32 has a central of order 4 and if we extend this group by an operator which transforms it according to an outer isomorphism then the resulting group has a central of order 4. If

this group admits an outer isomorphism under G we obtain a larger group which has a central of order 4. By continuing this process it results that either the central of G is of order 4 or that G is the direct product of a subgroup whose central is of this order and an abelian group of type $(1, 1, 1, \ldots)$, since only operators of order 2 can be commutative with every operator of a subgroup which involves operators of order 4 whose squares are unequal. To determine all the groups of order 2^m which come under the heading of the present article it is therefore only necessary to determine those whose central is of order 4.

It was noted above that every G contains invariantly both of the groups of order 16 which separately involve three and only three operators which are squares thereunder. As each of these subgroups has a group of inner isomorphisms of order 4 and cannot be transformed in more than 16 different ways under G it results that G involves a subgroup whose index does not exceed 16 composed of all of its operators which are commutative with each of the operators of one of these subgroups. This subgroup can involve only operators whose order divide 2 and it does not involve all the operators of order 2 in one of these subgroups of order 16. This proves the following theorem: *If a group of order 2^m involves three and only three operators which are squares thereunder then it contains an invariant abelian subgroup of index* 8 *and of type* $(1, 1, 1, \ldots)$ *and also an invariant abelian subgroup of this index and of type* $(2, 1, 1, \ldots)$.

To determine an upper limit of the order of G when its central is of order 4 it may be noted that each of the two given such groups of order 32 contains certain such subgroups of order 16 all of whose operators are left invariant only by operators of order 2 in G which are commutative with every operator of the subgroup of order 32 in which it appears. Since the direct product of this subgroup of order 32 and a group of order 2 cannot appear in such a G it results that when the central of G is of order 4 then the order of G cannot exceed 64. It remains therefore only to determine these groups of order 64. When the given subgroup of order 32 involves 24 operators of order 2 there are two and only two such groups which can be obtained by extending this subgroup by an operator which is commutative with every operator of a subgroup of order 16 whose operators have three distinct squares. One of these groups involves 48 operators of order 4 while the other involves only 40 such operators. When the given subgroup of order 32 involves only 16 operators of order 4 there results an additional group of order 64 which involves only 32 operators of order 4. Hence *there are seven groups of order 2^m, $m > 5$, which separately involve three and only three operators which are squares of operators contained therein.*

[1] G. A. Miller, *Amer. Jour. Math.*, **55**, 417–420 (1933).

LOGARITHMIC SOLUTIONS OF BIANCHI'S EQUATION

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Communicated July 20, 1933

The partial differential equation

$$\frac{\partial^n V}{\partial x_1 \partial x_2 \ldots \partial x_n} = MV$$

was discussed by Bianchi[1] with the aid of the methods of Riemann and Picard. The results were extended to a more general equation which was also studied by Niccoletti.[2] The original equation, for a constant value of M, was studied later by Sibirani[3] in connection with a generalization of the Bessel function and some partial differential equations were listed which could be solved with the aid of this function. The case in which M is constant has also been studied by Chaundy[4] who gives some solutions in the form of definite integrals which we wish to obtain here with the aid of Murphy's theorem.

Putting $M = 1$ we write $z = l_1 + l_2 + \ldots l_n$, where $l_1 = \log x_1$, $l_2 = \log x_2$, $\ldots$ $l_n = \log x_n$. The differential equation is then satisfied by $V = F(z)$ if

$$D^n F = e^z F \tag{1}$$

where D denotes the operator d/dz. The general solution of (1) may be written in the form[5]

$$F = \sum_{s=0}^{n-1} \frac{z^s}{s!} U_s(z) \tag{2}$$

where $U_0, U_1, U_2, \ldots U_{s-1}$ are functions which satisfy the equations

$$(D + E)^n U_{n-s} = e^z U_{n-s} \quad (s = 1, 2, \ldots n) \tag{3}$$

E being an operator such that $EU_{n-p} = U_{n-p+1}$ and U_s being defined to be zero when $s \geqq n$. The equation for U_{n-1} is

$$D^n U_{n-1} = e^z U_{n-1} \tag{4}$$

and we may write

$$U_{n-1} = C_{n-1}\left[1 + \frac{e^z}{(1!)^n} + \frac{e^{2z}}{(2!)^n} + \ldots\right] = c_{n-1} E_n(e^z) \tag{5}$$

where c_{n-1} is an arbitrary constant and $E_n(x)$ is Chaundy's notation for the function which Sibirani denotes by the symbol $L_{0,n}(nx)$, his definition of $L_{r,k}(x)$ being as follows:

$$L_{\nu,k}(x) = \sum_{s=0}^{\infty} \frac{(-1)^s \left(\frac{x}{k}\right)^{\nu+sk}}{s!\,\Gamma(\nu+s+1)\Gamma(2\nu+s+1)\ldots\Gamma(k\nu-\nu+s+1)} \qquad (6)$$

The equation for U_{n-2} is

$$D^n U_{n-2} + nD^{n-1}U_{n-1} = e^z U_{n-2}$$

and it is readily seen that

$$U_{n-2} = C_{n-2}E_n(e^z) - nc_{n-1}\left[\frac{e^z}{(1!)^n} + \left(1+\frac{1}{2}\right)\frac{e^{2z}}{(2!)^n} + \left(1+\frac{1}{2}+\frac{1}{3}\right)\frac{e^{3z}}{(3!)^n} + \ldots\right] \qquad (7)$$

where c_{n-2} is another arbitrary constant. The functions U_{n-3}, $U_{n-4}, \ldots$ may be found step by step and U_0 will involve n arbitrary constants.

Our aim now is to satisfy the partial differential equation by a series of type

$$V = \sum_{s=0}^{n-1} H_s U_s(z)$$

in which H_2 is a homogeneous polynomial of degree s in $l_1, l_2, \ldots l_n$. It is readily seen that the partial differential equation will be satisfied if

$$D_m H_p = \binom{n}{m} H_{p-m} \qquad \begin{matrix} p = 0, 1, \ldots n-1 \\ m = 1, 2, \ldots n-1 \end{matrix}$$

where the operators, $D_1, D_2, D_3, \ldots$ are defined by the equations

$$D_1 = \sum \frac{\partial}{\partial l_s}$$

$$D_2 = \sum \frac{\partial^2}{\partial l_r \partial l_s} \qquad r \neq s$$

$$D_3 = \sum \frac{\partial^3}{\partial l_r \partial l_s \partial l_t} \qquad r \neq s = t$$

and H_q is supposed to be zero when q is negative. From these equations it follows that the polynomials H_p all satisfy the system of partial differential equations

$$\frac{D_m H}{\binom{n}{m}} = \frac{D_1^m H}{\binom{n}{1}^m} \qquad m = 1, 2, \ldots n-1.$$

When $n = 3$ there is just one equation

$$\frac{\partial^2 H}{\partial l_1^2} + \frac{\partial^2 H}{\partial l_2^2} + \frac{\partial^2 H}{\partial l_3^2} = \frac{\partial^2 H}{\partial l_2 \partial l_3} + \frac{\partial^2 H}{\partial l_3 \partial l_1} + \frac{\partial^2 H}{\partial l_1 \partial l_2}$$

and the general solution is

$$H = F(l_1 + l_2 + l_3, l_1 + \omega l_2 + \omega^2 l_3) + G(l_1 + l_2 + l_3, l_1 + \omega^2 l_2 + \omega l_3)$$

where F and G are arbitrary functions of their arguments and $1 + \omega + \omega^2 = 0$.

The natural generalization of this for an arbitrary value of n is

$$H = \sum_{\sigma} F_{\sigma}(z, l_1 + \sigma l_2 + \sigma^2 l_3 + \ldots \sigma^{n-1} l_n)$$

where σ is a primitive root of the equation $x^n = 1$ and the summation extends over all the primitive roots. This form of solution is particularly useful because when H_{n-1} has been expressed in this form the corresponding expression for H_{n-m-1} is

$$H_{n-m-1} = \sum_{\sigma} \frac{\partial^m F_{\sigma}}{\partial z^m}.$$

The most interesting type of logarithmic solution of the partial differential equation is

$$V = E_n(e^z) \log \frac{x_2}{x_1}.$$

This solution may be generalized by writing $x_p - u_p(t)$ in place of x_p. Multiplying by $f(t)$, where $f(t)$ is an arbitrary analytic function and integrating round a contour enclosing just one root of $x_1 = u_1(t_1)$ and just one root of $x_2 = u_2(t_2)$, we have the solution

$$V = \frac{1}{2\pi i} \int E_n\{[x_1 - u_1(t)][x_2 - u_2(t)] \ldots [x_n - u_n(t)]\} f(t)dt \log \frac{x_2 - u_2(t)}{x_1 - u_1(t)}$$

$$= \int_h^{t_2} f(t)dt\, E_n\{\quad \text{“} \quad\}$$

by Murphy's theorem.[6] When $n = 3$ this result may be used to obtain Chaundy's solutions and many others.

[1] L. Bianchi, *Rome. Acc. L. Rend.* (5) **4**, 8–18 (1895).
[2] O. Niccoletti, *Ibid.* (5) **4**, 330–337 (1895).
[3] F. Sibirani, *Annali di Matematica* (3) **28**, 1–34 (1918).
[4] T. W. Chaundy, *Proc. London Math. Soc.* (2) **21**, 214 (1923).
[5] The functions U_s are found by Sibirani.
[6] H. Bateman, *Bull. Am. Math. Soc.*, **39**, 118–123 (1923).

UREASE ACTIVITY AS INFLUENCED BY OXIDATION AND REDUCTION

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Communicated August 2, 1933

During recent years there have appeared numerous descriptions of the effects of hydrocyanic acid, of hydrogen sulphide and especially of organic sulphydryl compounds in activating certain hydrolytic enzymes, for example, intracellular proteinases (cathepsins),[1] arginase[2] and pancreatic amylase.[3] Indeed, reduced glutathione has been called by Waldschmidt-Leitz the natural activator of animal cathepsin and of arginase. Recently Perlzweig[4] suggested that urease is associated with a sulphhydryl activator, possibly glutathione. This suggestion was based, in part, upon the old observation that cyanides and hydrogen sulphide reactivate crude urease preparations which have been "poisoned" by ions of heavy metals. Perlzweig assumes that the "poisoning" consists of the catalysis of the activator's oxidation and that the activator can be regenerated by the reducing action of cyanides and sulphides. Perlzweig also reported a weakening of the nitroprusside test (for sulphhydryl) accompanying the partial inactivation of crystalline urease by aeration.

Sumner[5] has given evidence that the protein-urease molecule itself contains a group which gives a nitroprusside test. Such groups are probably sulphhydryl. Furthermore Sumner's work indicates that highly active, recrystallized preparations of urease possess characteristic activities in the absence of supplementary material such as glutathione.

It has appeared to us that some of the phenomena in this field might be ascribed not to changes in a supplementary activator but to oxidation of and regeneration of the sulphhydryl groups of the enzyme itself. Accordingly, we have put this working hypothesis to a test with crystalline urease preparations and we shall report in this preliminary communication only sufficient of our experiments to indicate typical results.

It is a pleasure to acknowledge the coöperation of Dr. J. B. Sumner and Dr. D. B. Hand who enabled us to obtain highly active jackbean meal and advised us in the preparation of crystalline urease.

Crystalline urease was used throughout the investigation except when it was occasionally desired to compare its behavior with that of the usual crude preparations. The reactions with the various reagents studied were carried out at room temperature. Conductivity water was used throughout this work. For the determination of urease activity a mixture corresponding to 2.5 ml. of freshly prepared "crystalline urease" solution diluted with water or other reagents to 5.0 ml., was added to 25.0 ml.

1 *M* phosphate buffer, pH 7.5, 10.0 ml. water and 10.0 ml. 1 *M* urea at 30° and the solution (total volume, 50.0 ml.) was held in a bath at 30° for a definite time interval, after which 1.0 ml. was withdrawn and added to 1.0 ml. 0.25 *M* sulphuric acid. The ammonia was collected by the usual aeration technique and estimated by titration. In many individual tests the time intervals were one, five, ten, fifteen and twenty minutes; in some, only five and fifteen minutes. The "activities" referred to in this paper are expressed as the number of milligrams of ammonia nitrogen liberated by the action upon urea for 15 minutes of a control or reaction mixture prepared from 2.5 ml. of a crystalline urease solution, a quantity termed for the sake of discussion in this paper, one "unit portion."

The first group of experiments to be summarized here is concerned with the addition to crystalline urease preparations of reagents, such as cuprous oxide and phenylmercuric hydroxide, that are considered to form rapidly by reaction with sulphhydryl groups very characteristic *salts* or *derivatives* of substances containing such groups. It is important to note that none of these reagents has pronounced oxidizing properties under the experimental conditions used.

Thiol derivatives form with cuprous oxide cuprous mercaptides of the type, RSCu.[6] With mercurials such as phenylmercuric hydroxide, for example, they react as follows:

$$R—SH + HO—Hg—C_6H_5 \longrightarrow R—SHg—C_6H_5 + HOH.$$

If cupric copper were formed to any extent by self oxidation-reduction of cuprous copper no complication would ensue, for urease is not *rapidly* "poisoned" by cupric ions and is completely destroyed in their presence only after prolonged aeration (1.5 to 3.0 hours). To cite one of the experiments concerned with cuprous oxide: a urease solution, "activity 168," was shaken with 0.8 mg. red cuprous oxide, Cu_2O, per "unit portion." The activity of the resulting mixture or *of the clear solution obtained by filtration of the mixture* was determined at once and found to have been reduced to zero. The addition of excess hydrogen sulphide to either the mixture or the clear filtrate restored the urease activity to 83% of its original value. Potassium cyanide also restored the activity substantially. The effect of either hydrogen sulphide or cyanide upon the *initial solution* was to increase its activity slightly.

A typical observation with an organic mercurial may be described as follows. An aqueous urease solution of "activity 168" was diluted with phosphate buffer, pH 7.5, and shaken with 0.75 mg. benzyl mercuric chloride, $C_6H_5CH_2HgCl$, per "unit portion." The urease activity of the suspension was tested at once and found to be zero. The addition of an excess of hydrogen sulphide water restored the activity *fully* and the activity of the clear solution obtained by *filtration of the suspension* was

also very substantially restored by hydrogen sulphide or by potassium cyanide. Entirely analogous and equally striking were the results obtained with phenyl mercuric chloride, C_6H_5HgCl, hydroxide, C_6H_5HgOH and chloromethylmercuric chloride, $ClCH_2HgCl$.[7] In every case, the activity was completely "extinguished" by the mercurial and completely or almost completely restored by the action of hydrogen sulphide. The effect can hardly be ascribed to adsorption of the enzyme upon the suspended metallic derivatives, since the enzyme complexes were shown to be soluble and separable by filtration from the excess of metal compound used. As was anticipated, no significant inactivation was introduced by the use of a symmetrical *bis*-alkyl mercury compound, *bis*-iodomethyl mercury, ICH_2HgCH_2I.[7] This substance was tried principally to test the effect of its reactive iodine atoms. It must be stated, also, that iodide ions or *pure* iodoacetate were found equally unable to inactivate crystalline urease.

Aeration Experiments.—When crystalline urease solutions are vigorously aerated for 2.5 to 3.5 hours, either with or without the addition of phosphate buffer but in the absence of added catalysts such as cupric ions, their activity is decreased only about 10% and this small decrement is completely reversed by the addition of cyanides or hydrogen sulphide.

On the basis of the considerable amount of recorded work upon the oxygenation catalysis of sulphydryl compounds, it was thought that cupric ions would accelerate this oxidation. This was found to be true. It seems clear that the "poisoning" effect of cupric ions under the conditions used is largely due, not to the ions *per se*, but to their ability to accelerate catalytically oxygenation of the urease. The enzymic activity is completely and irreversibly destroyed when solutions of urease to which have been added cupric sulphate or cuprous oxide are aerated for 3.5 hours.

To an aqueous solution of crystalline urease, "activity 126," was added water, 1.0 ml. and 0.01% cupric sulfate, 0.25 ml. for each "unit portion." After the mixture was aerated for 0.5 hour, it was found to be only 17% as active as initially. The addition of hydrogen sulphide restored the activity to 43%. Cysteine (neutral) was somewhat less effective. If that portion of the inactivation that is reversible actually corresponds to an oxidation of the type, $2RSH \longrightarrow R—S—S—R$, then it is clear that the irreversible portion may correspond to a more deep seated change, possibly involving, for example, a more extensive oxidation of the sulphur atoms. This would be in harmony with results obtained after the employment of longer periods of aeration, where frequently it was found possible to restore with cyanide or hydrogen sulphide a considerable measure of activity after the enzyme had been rendered almost completely inactive.

The results of aerations carried out in the presence of phosphate buffer were especially consistent and reproducible. The following is a typical experiment. An aqueous solution of crystalline urease, "activity 242," was treated with 1.0 ml. 1 *M* phosphate buffer, pH 7.5, and 0.25 ml. 0.01% cupric sulfate for each "unit portion." The mixture was aerated for 30 minutes after which its activity was found to have been reduced to 40% of the initial value. Cyst*eine* (metal-free) added to a portion of the resulting mixture, unlike cyst*ine*, which had no effect, was found to restore the activity to a considerable degree and any of the substances, hydrogen sulphide, thioglycollic acid (neutralized), HS-glutathione (neutralized) was found to *reverse completely* the aeration effect, restoring the urease activity to the original value. It should be noted that thioglycollate, added to urease-phosphate-copper mixtures *before aeration*, exerted a pronounced protective influence against irreversible oxidative attack; after the activity of one such mixture was reduced by aeration for one hour to but a few per cent of the original, the addition of hydrogen sulphide water was found to effect a restoration of 80%. It is of considerable interest in this connection that although *crude* urease preparations are known to be sensitive to the action of certain metallic ions (for example, silver, mercuric), the activity of such preparations was found to be "protected" against catalyzed oxygenations of the type described above to an extent apparently beyond the protective capacity of thioglycollate with crystalline urease.

Hydrogen peroxide appears to induce no greater effect upon solutions of crystalline urease, at least for the first few hours of action, than uncatalyzed aeration and the small reduction in enzymic activity is completely reversed by the action of potassium cyanide.

The Effect of Iodine.—Urease activity is very readily destroyed by iodine. However, a careful study of the behavior of iodine with urease has revealed the conditions under which its action may be "controlled." The results of this phase of the investigation are best appreciated by a consideration of the detail of a few experiments typical of many.

To a solution in 1 *M* phosphate buffer (pH 7.5) of crystalline urease, the activity of which was 224, was added, per "unit portion," 0.40 ml. 0.0023 *N* iodine (in water) with an equivalent amount of potassium iodide. This resulted in a decrease in the enzymic activity to 2.5% of the original. Hydrogen sulphide water, added to a portion of the reaction mixture, restored the activity to 42% of the original; thioglycollate-ion, to 30%. In a similar experiment in which iodine was allowed to act in an aqueous urease solution, the activity was reduced to 4% of the original and restored by hydrogen sulphide to 64%.

To a second portion of the solution in phosphate buffer, activity 224 was added per unit portion 0.37 ml. 0.0012 *N* iodine with an equivalent

amount of potassium iodide. The activity was reduced thereby to 74% of its initial value. The addition of hydrogen sulphide restored the activity completely.

Several additional features of the iodine work deserve comment. It seems very significant that potassium cyanide, unlike hydrogen sulphide, was found incapable of restoring the activity of crystalline urease specimens "poisoned" by iodine. This is not surprising when the few facts regarding protein structure are taken into consideration. Iodine would be expected to react rapidly, not only with thiol groups by oxidation but also with other characteristic groupings in the protein molecule (for example, $—NH_2$, —NH—) by substitution, etc. The situation is doubtless much more complex than that concerned with "mild" oxygenations. The subject cannot be elaborated here. It should be pointed out, however, that the more drastic action of iodine might involve a deep seated change due to permanent substitutions in the protein molecule as well as oxidation of thiol sulphur beyond the dithio stage, in which case irreversible inactivation of a labile, highly specific protein-enzyme molecule would be anticipated. On the other hand, more superficial substitutions in the protein molecule accompanying the oxidation of thiol to dithio might be wholly reversed by such a reagent as hydrogen sulphide but not necessarily by cyanide. It has long been known, for example, that the action of potassium cyanide upon certain halogenoamino (N-halogen) compounds may involve a change more complex than the substitution for halogen of hydrogen atoms which is characteristic of a number of other reducing agents. These remarks are admittedly speculative as applied to the present problem; nevertheless, they are offered for what suggestive value they may possess.

Another point of interest concerned with the work with iodine may be summed up in the statement that we have thus far failed to discover any specific effect resulting from a change in the concentration of iodide-ion over a wide range in conjunction with the use of a given quantity of iodine favorable for the reversible inactivation of urease. That is, the state of the iodine–iodide system as a strictly reversible system, seems unimportant for its application to the present problem. This is in no respect inconsistent with our present knowledge of the oxidation-reduction behavior of mercaptans and dithio compounds.

[1] Willstätter, R., and Grassmann, W., *Z. physiol. Chem.*, **138**, 184 (1924); Waldschmidt-Leitz, E., and co-workers, *Ibid.*, **188**, 17 (1929–30); *Ibid.*, **198**, 260 (1931); Grassmann, W., Schoenebeck, O., and Ebeler, H., *Ibid.*, **194**, 124 (1930–31).

[2] Salaskin, S., and Solowjew, L., *Ibid.*, **200**, 259 (1931); Waldschmidt Leitz, E., Schäffner, A., and Kocholaty, W., *Naturwissenschaften*, **19**, 964 (1931).

[3] Pringsheim, H., Borchadt, H., and Hupfer, H., *Biochem. Z.*, **238**, 476 (1931); *Ibid.*, **259**, 134 (1933).

[4] Perlzweig, W. A., *Science*, **76**, 435 (1932); *Proc. Am. Soc. Biol. Chemists*, **77** (1933).

[5] Sumner, J. B., *J. Biol. Chem.*, **69**, 435 (1926); *Ergebnisse der Enzymforschung*, **1**, 295–301, Leipzig (1932); Sumner, J. B., and Poland, L. O., *Proc. Soc. Exptl. Biol. Med.*, **30**, 553 (1933).

[6] Hopkins, F. G., *J. Biol. Chem.*, **84**, 269 (1929); Pirie, N. W., *Biochem. J.*, **25**, 614 (1931).

[7] Hellerman, L., and Newman, M. D., *J. Am. Chem. Soc.*, **54**, 2859 (1932).

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*THE ELECTRON-DIFFRACTION INVESTIGATION OF THE STRUCTURE OF MOLECULES OF METHYL AZIDE AND CARBON SUBOXIDE**

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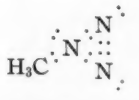
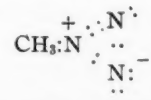
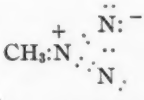
Communicated August 8, 1933

We have applied the electron-diffraction method of studying the structure of gas molecules, developed by Mark and Wierl and briefly described by us in a recent paper,[1] in the determination of the atomic configuration and interatomic distances in methyl azide and carbon suboxide molecules. The results of the investigation are given in the following paragraphs, together with a discussion of the information they provide regarding the electronic structure of these molecules.

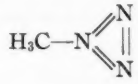
Methyl Azide.—Two distinct structural formulas have been proposed for methyl azide, CH_3N_3; in one the nitrogen atoms form a three-membered ring, and in the other an open chain. It is known that the azide ion has a linear configuration in crystals of sodium azide and potassium azide,[2] but it has sometimes been contended that this configuration may not be retained in organic azides. Other methods of investigation have not led to a decision, the electric moment measurements, for example, being compatible with either structure.[3] Our results show the azide group in methyl azide to be linear.

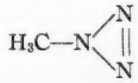
Each of five electron-diffraction photographs of methyl azide shows only one apparent maximum within the 6° angular range allowed by the dimensions of the apparatus. This maximum is exceedingly sharp and distinct, and is susceptible to measurements of greater than the usual accuracy. The average measured value for $\frac{\sin \theta/2}{\lambda}$ for it is 0.470, and that for the well-defined apparent intensity minimum is 0.371. These values are to be compared with intensity curves calculated for various models, as described in Reference 1.

Reasonable Lewis electronic structures in which the nitrogen atoms form a ring are the following:

 I  II  III.

Since these structures contain the same total number of bonds and none of the atoms is doubly charged, we would expect that the molecule in its normal state would resonate among all three structures, their contributions being about equal. The inclusion of II and III in the group of resonating structures would require the methyl group to be coplanar with the ring. We shall call this structure A.

A.  Coplanar Complete resonance among I, II and III

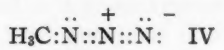
B.  Coplanar Structure I alone

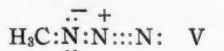
C. Same as B, but with C—N to N—N bond angles 133° 30′

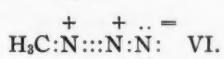
D. Same as C, but with bond angles 109° 28′.

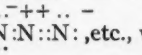
Calculations were also made for the less probable structures B, C and D (Fig. 1*a*). In each of these the interatomic distances are given by the type of bond and the atoms involved:[4] 1.47 Å for C—N, 1.40 Å for N—N, 1.26 Å for N=N and 1.06 Å for C—H. The four carbon bonds are assumed to make angles of 109° 28′ with each other. Interference between hydrogen and nitrogen atoms is neglected.

Reasonable electronic structures for a nitrogen chain are the following:

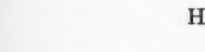
 IV

 V

 VI.

(In addition to these important structures, structures of the type  , etc., would contribute to a small amount, without changing the interatomic distances or bond angles.) We expect structures IV and V to contribute equally to the normal state of the molecule, since for them the total number of bonding electron pairs is the same and the arrangement of formal charges is nearly the same. Structure VI should be considerably less important because of the juxtaposition of atoms with charges of the same sign. Inasmuch as each of these structures corresponds to a col-

linear arrangement of the nitrogen atoms in the azide group, the only bond angle varied in the structures for which calculations were made is that between the C—N bond and the axis of the azide group. The structures considered are the following, the corresponding intensity curves being given in figure 1*b*:

E.	$H_3C—N{\equiv}N{\equiv}N$	Collinear	Complete resonance among IV, V and VI
F.		Bond angle 125°	Structure IV
G.		Bond angle 125°	Partial resonance between IV and V, with $N{\equiv\!=}N$ = 1.16 Å
H—100°		Bond angle as given	Complete resonance between IV and V
H—125°			
H—129° 10′			
H—136° 41′			
H—150°			

The interatomic distances used are those mentioned above, and $N{\equiv}N$ = 1.10 Å.

The qualitative comparison of the calculated curves of figures 1*a* and 1*b* and the observed photographs, showing a sharp maximum and minimum, leads immediately to the elimination of E. The quantitative comparison may be conveniently made by combining the assumed N=N interatomic distance of 1.26 Å with the observed $\frac{\sin\theta/2}{\lambda}$ values for the minimum and maximum to obtain "observed" values of $x = 2{\cdot}1.26{\cdot}\frac{\sin\theta/2}{\lambda}$. This procedure is accepted as reliable because the atomic radii used have been verified by comparison with data for a large number of substances; and any analysis taking into account the effect of resonance on interatomic distance which gave values appreciably different from those obtained with the aid of the table would be rejected. The narrow shaded regions in the figures correspond to the observed position of the maximum with limits of error of ±2%. The shaded region for the first minimum extends from the "observed" value of x to a value 10% smaller, inasmuch as we have found that for other substances investigated the visually estimated ring diameter for the first minimum is uniformly about 8 or 10% larger than

the value given by calculation. Any structure for which the calculated maximum and minimum lie outside of the shaded regions is definitely eliminated. It is seen that all of the ring-type structures are ruled out, as

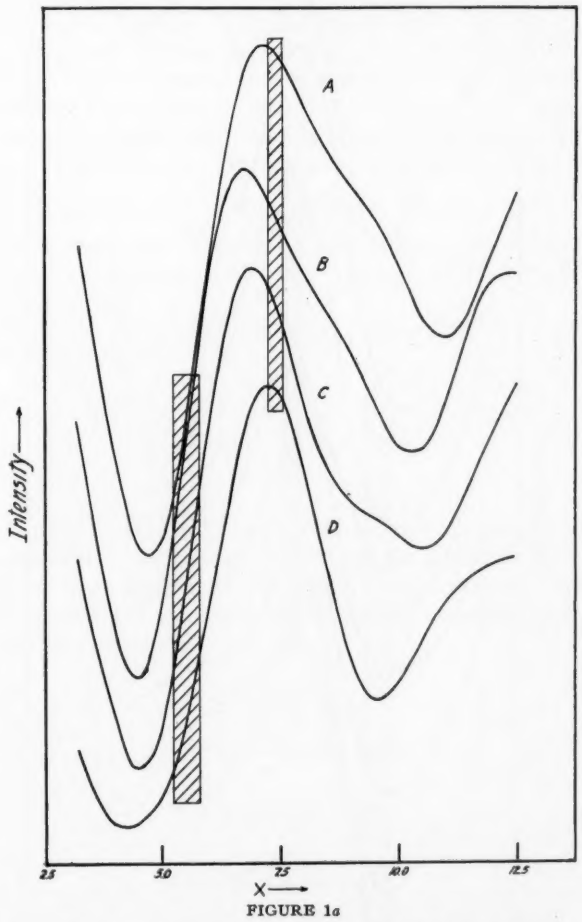


FIGURE 1a

Theoretical intensity curves for cyclic structures of methyl azide.

well as F and H-100°. This leaves G, representing partial resonance between IV and V, and H-125° to H-150°, representing complete resonance between these structures. While the experimental agreement with G is about as good as with H, the probability of complete resonance between IV and V is very high, since the corresponding structures contribute

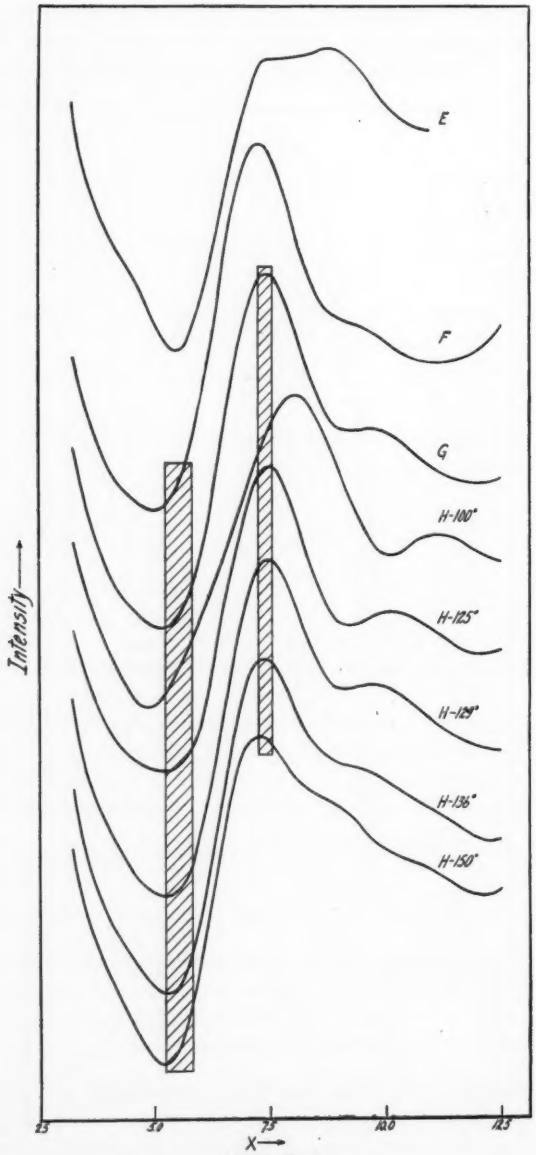
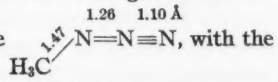
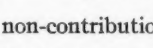
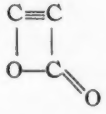
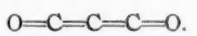


FIGURE 1b

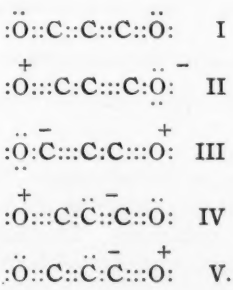
Theoretical intensity curves for chain structures of methyl azide.

equally in the cases of CO_2 and N_2O. The atomic arrangement of the normal state of methyl azide is thus found to be  , with the bond angle equal to 135° ± 15°. The uncertainty in the experimental verification of the interatomic distances is not larger than ±2%. The value found for the bond angle is close to that for a tetrahedral atom, 125°. The arrangement corresponds to resonance of the molecule among the two principal structures IV and V, with structure VI making no significant contribution. This supports the suggestion, originally advanced[5] to account for the non-contribution of the structure  to the normal state of nitrous oxide, that no important part in the normal state of molecules is played by structures containing adjacent atoms with electrical charges of the same sign.

Carbon Suboxide.—Both a ring and a chain structure have been proposed also for carbon suboxide:

 and 

The ring structure is extremely improbable because the bond angles for acetylene-type carbon atoms should be 180°, and the strains involved in the four-membered ring would make the molecule very unstable. We have hence not considered this structure. The most important electronic structures for the chain model are



Of these the first three lead to a completely collinear arrangement of the atoms, so that we may safely assume all bond angles to be 180°. Resonance among all five structures leads to a model with distances corresponding to triple bonds throughout (C≡C = 1.22 Å, C≡O = 1.13 Å). The same atomic configuration results from complete resonance among I, II

and III only. Structure I alone leads to C=C = 1.38 Å and C=O = 1.28 Å, whereas partial resonance of I with II and III would lead to intermediate values, the ratio of the carbon-carbon to carbon-oxygen distance remaining constant (equal to 1.08). Curve *A* in figure 2 consequently

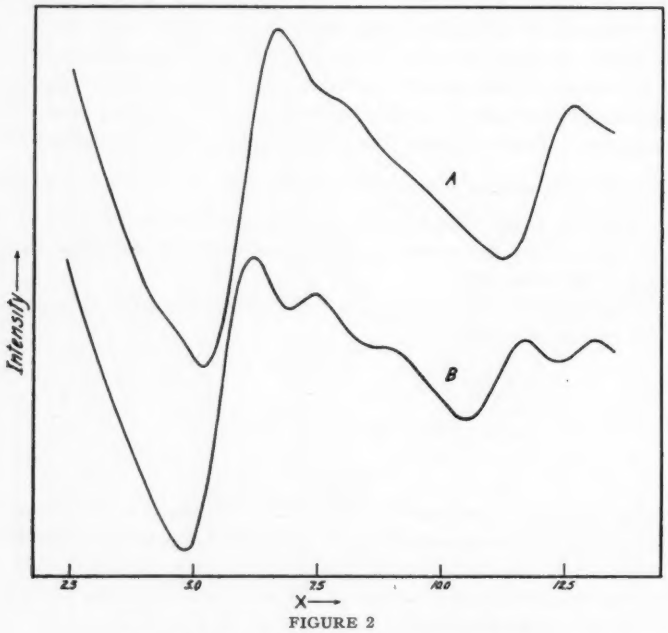


FIGURE 2

Theoretical intensity curves for carbon suboxide.

represents all of these models, the parameter x being equal to the product of $\frac{\sin \theta/2}{\lambda}$ and the carbon-oxygen distance. Curve *B* represents resonance of I with IV and V.

The photographs show two distinct rings. There is no indication of the step-wise decrease in intensity following the first maximum required by *B;* therefore this model is eliminated. The quantitative comparison of the two minima and two maxima with curve *A* is given in table 1.

TABLE 1

	$\frac{\sin \theta/2}{\lambda}$	x	a
1st min.	0.367	5.18	1.12
1st max.	0.438	6.66	1.21
2nd min.	0.756	11.22	1.18
2nd max.	0.849	12.70	1.19

The agreement in the values of the carbon-oxygen distance a is satisfactory, taking into account the usual 8 or 10% deviation shown by the first minimum, and provides strong support for the model. The averaged value of a is 1.193 Å (neglecting the first minimum), which becomes 1.20 Å when the small density correction required by comparison with the carbon tetrachloride standard photographs is made. The interatomic distances hence have the values

$$\text{C to O} = 1.20 \pm 0.02 \text{ Å}, \qquad \text{C to C} = 1.30 \pm 0.02 \text{ Å},$$

which are just midway between the double-bond and triple-bond values. Consequently the most important contribution to the normal state of the molecule is made by structure I; there is, however, incomplete resonance to structures II and III, to an amount sufficient to decrease the interatomic distances appreciably. The elimination of model B indicates that structures IV and V contribute still less than II and III. The bonds in the molecule have properties intermediate between double and triple bonds, and the normal state might be conveniently represented by the formula O=C=C=C=O.

We are indebted to Dr. G. W. Wheland for preparing the methyl azide and carbon suboxide used in the experiments.

* Contribution from Gates Chemical Laboratory, California Institute of Technology, No. 353.

[1] Brockway, L. O., and Pauling, L., these PROCEEDINGS, **19**, 68 (1933).

[2] Hendricks, S. B., and Pauling, L., *Jour. Amer. Chem. Soc.*, **47**, 2904 (1925).

[3] Sidgwick, N. V., Sutton, L. E., and Thomas, W., *J. Chem. Soc.*, **406** (1933).

[4] Pauling, L., these PROCEEDINGS, **18**, 293 (1932).

[5] Pauling, L., *Ibid.*, **18**, 498 (1932).

THE ELECTRON-DIFFRACTION INVESTIGATION OF THE MOLECULAR STRUCTURE OF CYANOGEN AND DIACETYLENE (WITH A NOTE ON CHLORINE DIOXIDE)*

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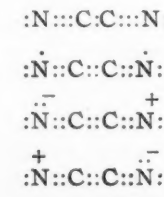
Communicated August 8, 1933

The structures of cyanogen and diacetylene have been previously investigated by the electron-diffraction method by Wierl,[1] who reported non-linear models with 150° bond angles for both molecules. The improbability of this conclusion, which is incompatible with all other evidence for the angle between a triple and a single bond on the same carbon atom, led to a reinvestigation of these compounds by the electron-diffraction method.

The models for which theoretical intensity curves were calculated were chosen from a consideration of the electronic structures corresponding to low energy values for the molecule. Those for cyanogen may be represented as follows:



I

II

III

IV.

The probable atomic configurations may be chosen on the basis of Pauling's[2] discussion of resonance between Lewis electronic structures. The contribution of I to the resonating structure is as important as that of any single structure above because of the two strong bonds which it contains; therefore, the only necessary consideration is the effect of combining the others with I. II, III and IV would tend to decrease the carbon-carbon distance toward that of a double bond. Of these three II is the most important because each atom is electrically neutral and the molecule has a lower energy value than III or IV has.

Four theoretical intensity curves have been calculated, in which the ratio of carbon-carbon to carbon-nitrogen distance has the values 1.54/1.16, 1.46/1.16, 1.42/1.16 and 1.38/1.16. In the figures the curves are designated with the numerators of these fractions. The first corresponds to I alone, the fourth to complete resonance of I with the others, and the second and third to two stages of partial resonance. The most important qualitative feature of these curves is the disappearance of the second maximum in going from the first to the fourth curve.

The photographs of cyanogen are characterized by a sharp first ring with

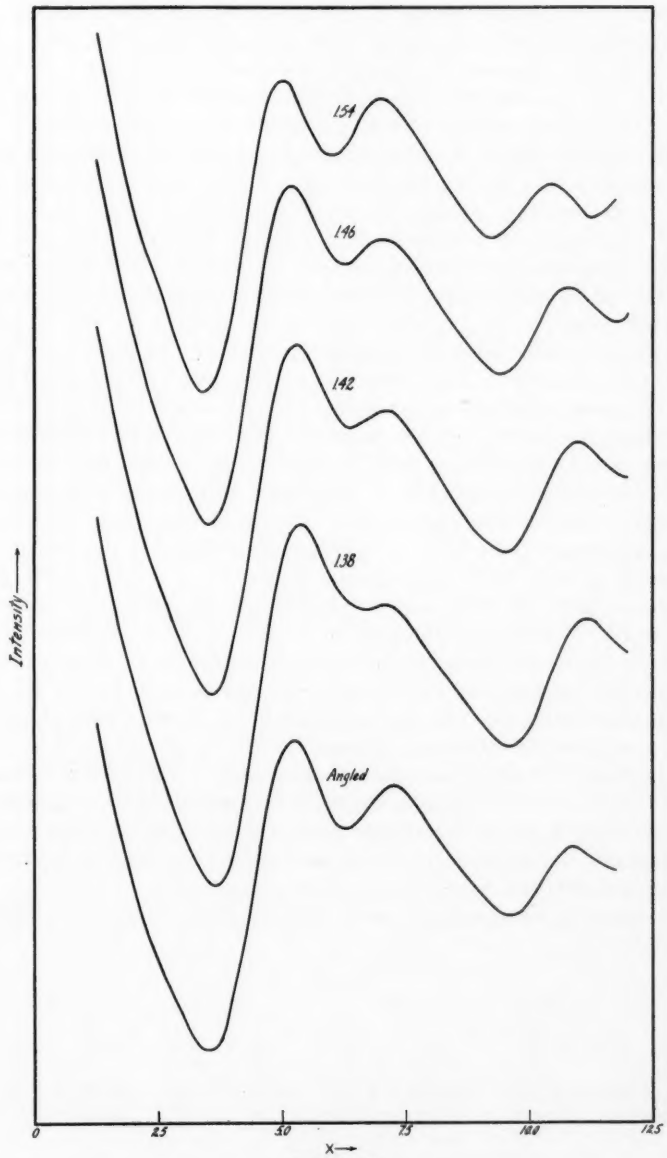


FIGURE 1

Theoretical intensity curves for cyanogen.

a broad "shelf" slowly falling off toward the outside edge. This is followed further out by a maximum which is sharply defined on both sides. This qualitative appearance eliminates the single-bond model I since the result of the investigation of sulphur hexafluoride[3] indicates that such a double maximum is duplicated in the appearance of the photograph.

The reproductions of the photographs obtained by Wierl show the same characteristics and lead to the elimination of the same model. His unwillingness, however, to alter the two bond distances from the chosen values, 1.52 and 1.20, led him to relinquish the linearity of the molecule and to assume an angle of 150° between the carbon-carbon and carbon-nitrogen bonds, and he claimed thereby to find satisfactory agreement with the photographs.

Three objections must be brought against the conclusion of Wierl. The first is that in no other case has a compound containing a triple-bonded carbon atom been measured by any method and found to have the single bond not in a straight line with the triple bond. The second is that in view of the resonance possibilities suggested by Pauling and for which additional evidence is furnished by the results of this investigation there is no need to consider non-linear models. The third objection is to be found in the appearance of the fifth curve in the illustration. In this the second maximum is only a little less prominent than in the first curve. The fifth curve was calculated from the 150° model but using the bond distances 1.54 and 1.16 taken from the table of covalent radii.[2] The discrepancy with Wierl's curve is probably due to his use of somewhat different bond distances which appear to correspond to the case of diacetylene. His use of the same curves for cyanogen and diacetylene is open to the objection that it neglects the difference between the carbon-nitrogen and carbon-carbon triple-bond distances in the two compounds. This leads to different values for the central carbon-carbon bond distances in the two compounds, a result which is highly improbable because of the identical electronic arrangement. Wierl's support of the model with the 150° angle is, therefore, the result of incorrect interpretation of the photographs.

The quantitative comparison of the photographs is shown in the following table:

TABLE 1

	$\frac{\sin\theta/2}{\lambda}$	1.46		1.42		1.38	
		x	a	x	a	x	a
1st min.	0.352	4.77	1.08	4.85	1.10	4.88	1.10
1st max.	0.427	6.45	1.20	6.55	1.22	6.63	1.24
2nd min.	0.718	10.63	1.18	10.82	1.20	10.85	1.21
2nd max.	0.831	12.03+	1.15	12.23	1.17	12.45	1.19

The values in the first column are the averages from two photographs. The x-values are taken from the theoretical curves for the respective

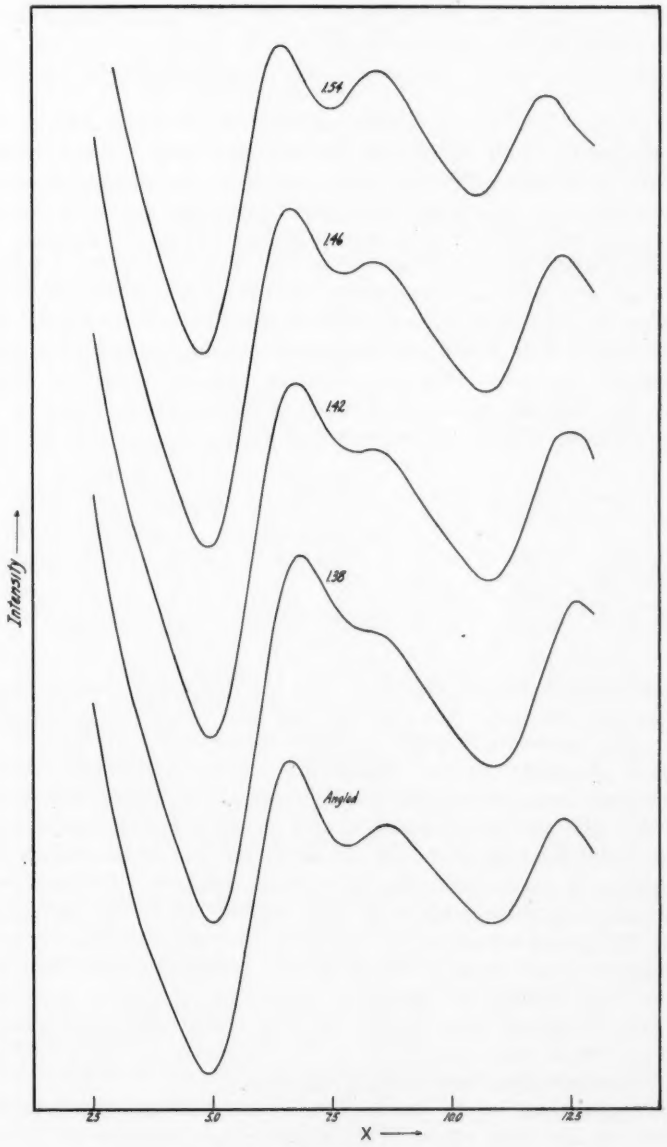


FIGURE 2

Theoretical intensity curves for diacetylene.

models. The a-values correspond to the carbon-nitrogen triple-bond distance for which 1.16 is the expected value.

Comparison with the standard CCl_4 photographs shows that no density correction is to be applied.

The lack of complete agreement among all of the a-values for a single model is expected on the basis of the St. John effect. I have found in working on electron-diffraction photographs that the measurement of an asymmetric ring (such as one of a pair of rings close together or one ring with a very different rate of decline in apparent density on the inside and outside) always gives a value for the interatomic distance which is different from that calculated from any sharp, well-defined ring. The discrepancy between the position of an asymmetric spectral line as measured subjectively with a filar micrometer and objectively by a recording microphotometer has been thoroughly investigated by St. John and Ware.[4] They show that the apparent maximum of such a line measured subjectively is always shifted toward the side of greater contrast, and the effect

TABLE 2

	$\frac{\sin\theta/2}{\lambda}$	1.46		1.42		1.38	
		x	a	x	a	x	a
1st min.	0.356	4.90	1.10	4.90	1.10	5.03	1.12
1st max.	0.430	6.63	1.23	6.70	1.24	6.80	1.26
2nd min.	0.753	10.75	1.14	10.80	1.14	10.90	1.15
2nd max.	0.833	12.32	1.18	12.53	1.20	12.65	1.21

on the measurement of a doublet is such that the apparent separation is greater than the true. The cause for this shift lies in the psychological effect of the difference in brightness of the background on the two sides of the line. A particularly clear example of this effect in the measurement of electron-diffraction photographs has been found in the case of sulphur hexafluoride; here the inner component of a double ring is shifted in by six per cent and the outer is shifted out by six per cent as determined by a comparison of the corresponding interatomic distances with those calculated from two sharp single rings lying farther out in the photograph. While the general appearance of the asymmetric characteristics of such a photograph is very useful in the qualitative comparison with theoretical curves, good quantitative agreement among all the rings is impossible. The exact size of the correction to be applied cannot be predicted, but an allowance for the effect may be made by determining the interatomic distance from the sharply defined single rings only.

The measurement of the position of the minima on the photograph also involves the St. John effect. This is particularly apparent in the first minimum because of the extreme density of the central image. Measurement of the photographs of fifteen different compounds taken with various

intensities shows that the position of the first minimum is always shifted out by from eight to ten per cent.

In table 1 for cyanogen the first minimum shows the usual deviation. The first maximum is of the type which shows the St. John effect and hence the corresponding a-value is larger than the correct one. The sharp second maximum affords a correct estimation of the value of a. The "1.38" model is not satisfactory, but the other two can scarcely be distinguished. The most probable values are chosen as follows:

$$\text{C—N} = 1.16 \pm 0.02 \text{ Å}$$
$$\text{C—C} = 1.43 \pm 0.03 \text{ Å}.$$

These results show that the single-bond models are somewhat more important than the double-bond models in the resonating structure which represents the structure of cyanogen.

The theoretical discussion for diacetylene is exactly the same as for cyanogen. The models calculated again vary from 1.54 to 1.38 for the central bond distance while the triple-bond distance is 1.22 and the carbon-hydrogen bond is 1.06 throughout. It will be noticed in figure 2 that the subsidiary maximum in the angled model is relatively less important than in the case of cyanogen.

The qualitative comparison as before eliminates the single-bond model; the quantitative comparison is indicated in table 2.

Comparison with the intensity standards indicates about a 1% correction. The values calculated from the sharp second maximum become 1.19, 1.21 and 1.22, respectively. The most probable values are

$$\text{C—C at end} = 1.21 \pm 0.02 \text{ Å}$$
$$\text{C—C in center} = 1.43 \pm 0.03 \text{ Å}.$$

TABLE 3

$\frac{\sin\theta/2}{\lambda}$ OBSERVED	MODEL I 180° x	MODEL I 180° a	MODEL II 150° x	MODEL II 150° a	MODEL III 126° x	MODEL III 126° a	MODEL IV 110° x	MODEL IV 110° a	MODEL V 90° x	MODEL V 90° a	MODEL VI 80° x	MODEL VI 80° a
1st min. 0.282	4.72	1.335	4.75	1.343	4.60	1.300	4.35	1.230	4.25	1.201	4.63	1.309
1st max. 0.397	7.55	1.514	7.60	1.524	7.80	1.564	7.92	1.588	7.72	1.548	7.90	1.584
2nd min. 0.556	11.13	1.592	11.12	1.590	10.65	1.524	10.80	1.545	11.20	1.603	11.15	1.595
2nd max. 0.737	13.85	1.496	13.90	1.502	14.30	1.545	13.90	1.502	14.15	1.528	14.30	1.545
3rd min. 0.921	17.45	1.508	17.40	1.504	17.10	1.478	17.40	1.504	17.05	1.474	17.30	1.495
Mean value		1.528		1.530		1.528		1.535		1.538		1.555
Average deviation		0.033		0.030		0.027		0.032		0.037		0.035

Final value Cl—O = 1.53 ± 0.03 Å

This agrees satisfactorily with the result for cyanogen. The single bond model for diacetylene, too, makes a greater contribution to the final structure than that of the models containing only six bonding electron pairs.

I wish to express my appreciation to Professor Pauling at whose suggestion this investigation was undertaken, and to Dr. G. W. Wheland for the preparation of the diacetylene.

NOTE ON CHLORINE DIOXIDE

The experimental value for the chlorine-oxygen separation in ClO_2, which was published recently in these PROCEEDINGS,[5] is 0.05 Å too large because of a numerical error which occurred in the computation of the $\frac{\sin \theta/2}{\lambda}$ values from the observed ring diameters. Table 3 contains the corrected $\frac{\sin \theta/2}{\lambda}$ and a-values. The most probable value based upon this table is Cl—O = 1.53 ± 0.03 Å.

Although the combination of electron-pair with three-electron bond is surmised to produce a chlorine-oxygen separation of about 1.57 Å, which is midway between the single-bond and double-bond value, some deviation from this may well occur; and, inasmuch as it is impossible to write for ClO_2 any structure with double bonds which does not involve high-lying energy values, the observed separation of 1.53 Å can be explained only on the basis of the discussion previously given, so that this corrected result still substantiates the type of resonance represented by the three-electron bond. The separation in the combined bond is evidently nearer to that of the double-bond (1.48 Å) than to the single-bond distance (1.65 Å).

* Contribution from Gates Chemical Laboratory, California Institute of Technology, No. 354.

[1] Wierl, R., *Ann. Phys.*, **13**, 453 (1932).
[2] Pauling, Linus, these PROCEEDINGS, **18**, 293 (1932).
[3] Brockway, L. O., and Pauling, Linus, *Ibid.*, **19**, 68 (1933).
[4] St. John, C. E., and Ware, L. W., *Astrophys. Jour.*, **44**, 35 (1916).
[5] Brockway, L. O., these PROCEEDINGS, **19**, 303 (1933).

OXIDATION-REDUCTION POTENTIAL OF ASCORBIC ACID (VITAMIN C)

BY HENRY BORSOOK AND GEOFFREY KEIGHLEY

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Communicated August 4, 1933

The measurement of the oxidation-reduction potential of ascorbic acid (Vitamin C) is desirable in the elucidation of its physiological action. Two attempts have been made to measure this potential. Georgescu[1] concluded that he had obtained a thermodynamically reversible potential at pH 6.9, and 7.0 at 20°C. From the data presented, however, it is not possible to accept this interpretation without reservation. For instance at pH 6.9 the potential difference observed between 5 per cent and 80 per cent oxidation of the vitamin was only 22 millivolts where it should have been 55 millivolts. At pH 7.0 no potential difference was observed between 12.5 per cent and 75 per cent oxidation where the theoretical difference is 39 millivolts. Moreover the values of the mid points of the two curves given at pH 6.9 and pH 7.0 lead to the exceedingly improbable value for $-\frac{dE}{dpH}$ of 240 millivolts.

More recently Laki[2] working at pH 7.33 reported his failure to confirm the findings of Georgescu. Laki's observations indicated that the potential was proportional only to the concentration of the reduced form.

By working through a range of hydrogen-ion concentrations from pH 2.0 to pH 5.75 we have succeeded in measuring thermodynamically reversible potentials with ascorbic acid. The failure of the two previous workers is to be attributed, we believe, chiefly to their having worked for the most part near neutrality where the oxidized form of ascorbic acid decomposes very rapidly.

The measurements were carried out at 35.5°C. with the vacuum technique previously described[3] except that no reversibly oxidizable dye was added. For the preparations of the various mixtures of oxidized and reduced ascorbic acid the reduced form was oxidized with iodine. The ascorbic acid used, kindly given us by Professor von Szent-Györgyi, gave a titration value with iodine of 100% on the basis of 1 molecule of reduced ascorbic acid yielding on oxidation two hydrogen ions.

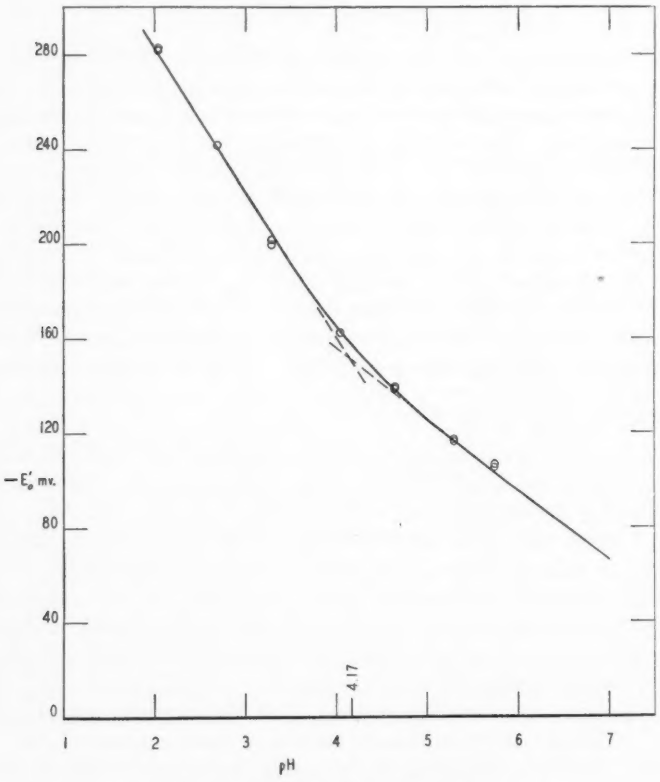
The electrode equation was obtained by the derivation described earlier.[3] For the mechanism

$$\left[\begin{matrix}\text{Reduced}\\ \text{Ascorbic Acid}\end{matrix}\right]^{-} \longrightarrow \left[\begin{matrix}\text{Oxidized}\\ \text{Ascorbic Acid}\end{matrix}\right]^{-} + 2H^{+} + 2(e)$$

the equation is

$$E_{obs} = \tilde{E} + \frac{RT}{F}\,\mathrm{pH} + \frac{RT}{2F}\ln\frac{[\mathrm{Red}]}{[\mathrm{Ox}]} + \frac{RT}{2F}\ln\frac{K_R}{K_o} + \frac{RT}{2F}\ln\frac{K_o + [H^+]}{K_R + [H^+]}$$

where [Red] and [Ox] indicate the total concentration of reduced and oxidized forms, respectively, and K_R and K_o the first dissociation constants of



Curve is theoretical for $pK_R = 4.17$, $pK_o = 9.0$
O are experimental values

the reduced and oxidized forms considered as monovalent acids. For simplicity we have ignored the second dissociation constants. Birch and Harris[4] found the value of pK_{R_1} to be about 4.17. By a colorimetric titration procedure we have found the value of pK_o to be approximately 9.0. The value of n was taken as 2, which was indicated by the results of the iodine titration, and corroborated by the agreement between the observed

and calculated results in table 1. With these values of the dissociation constants and of n, and a mean value of $\tilde{E}$ of -0.553 volt the graph presented was constructed.

Table 1 shows that at any given pH between 2.0 and 5.75 the potential is proportional to the ratio of the concentrations of reduced and oxidized forms. The observed and calculated potential differences agree whether the concentraton of reduced form is held constant and the concentration of the oxidized form changed, or the reverse, or whether both are varied.

TABLE 1

REDUCTION POTENTIALS AT 35.5°C. OF DIFFERENT MIXTURES OF REDUCED AND OXIDIZED ASCORBIC ACID AT DIFFERENT HYDROGEN-ION CONCENTRATIONS

pH	CONCENTRATION OF REDUCED FORM $\times 10^3$ MOLS	CONCENTRATION OF OXIDIZED FORM $\times 10^3$ MOLS	RATIO $\frac{[RED]}{[OX]}$	CALCULATED P.D. = $0.0306 \log \frac{[RED]}{[OX]}$ MV.	P.D. OBSERVED MV.	E'_o OBSERVED MV.	E'_o CALCULATED MV.
2.04	2.43	0.504	4.82	18	17	−0.283	−0.281
2.04	2.43	2.12	1.20			−0.281	
2.68	0.74	1.38	0.54	18	18	−0.242	−0.242
2.68	2.96	1.38	2.14			−0.242	
3.30	0.72	1.41	0.51	18	16	−0.202	−0.204
3.30	2.87	1.41	2.03			−0.200	
4.06	0.73	1.40	0.52	18	19	−0.163	−0.164
4.06	2.93	1.40	2.07			−0.163	
4.65	0.75	1.38	0.55	18	17	−0.139	−0.139
4.65	3.01	1.38	2.18			−0.140	
5.31	1.91	1.82	1.05	18	17	−0.117	−0.117
5.31	1.91	0.45	4.2			−0.118	
5.75	4.55	0.56	8.18	37	38	−0.106	−0.104
5.75	1.14	2.23	0.51			−0.107	

In table 2 the values given were taken from the graph shown. Our last reliable experimental determination was at pH 5.75. At more alkaline

TABLE 2

ASCORBIC ACID. RELATION BETWEEN $E'_o \left(\frac{[Red]}{[Ox]} = 1\right)$ AND pH

pH	E'_o
2.0	−0.283
2.5	−0.252
3.0	−0.223
3.5	−0.193
4.0	−0.166
4.5	−0.145
5.0	−0.127
6.0	−0.096
7.0	−0.066

hydrogen-ion concentrations the rate of decomposition of the oxidized form was too rapid for any reliable measurements to be made. The curve and

the figures in table 2 beyond pH 5.75 therefore are extrapolations unsupported at present by any direct experimental data except the measurement of the dissociation constant of the oxidized form.

The potentials obtained elucidate the earlier observations on the stability of Vitamin C, its behavior toward various oxidizing and reducing agents and its physiological action. These considerations will be presented in detail later.

We wish to acknowledge our indebtedness to Professor A. von Szent-Györgyi for his gift of the specimen of pure ascorbic acid used in this investigation, and to thank him heartily for his generosity.

Summary.—1. Data regarding the thermodynamically reversible reduction potentials of ascorbic acid (Vitamin C) are presented.

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